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A NOTE ON THE RATE OF MORPHOGENETIC MOVEMENT IN THE SLIME MOLD, DICTYOSTELIUM DISCOIDEUM[1]

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(Received for publication on July 19, 1945)

The work presented here, although concerned with one of the lower organisms, closely parallels the investigations of Tyler on sea urchin embryos. Tyler (1933, 1935) produced by fission and fusion of sea urchin embryos dwarf or "half" and giant embryos. When comparing the rates of development of dwarf, normal, and giant embryos he found that the larger the embryo, the faster the rate of development. The rate of oxygen consumption per unit mass was found to be identical for all size embryos, and therefore the slower the rate of development the greater is the total energy per unit mass required for development. In other words, the smaller the sea urchin embryo, the more energy per unit mass is required for its development.

If this work of Tyler's is to be extended there are two points that must be more fully satisfied. (*a*) In the first place, as Needham (1942) points out, Tyler has been unable to make a separation in his experiments between growth and morphogenesis.[4] Tyler (1942) apparently is aware of this problem, for he states that "there is no evidence as yet that the form changes can occur without growth." (b) Tyler is also limited in the number of organisms of different sizes he can test. He is comparing the rate of morphogenetic movement to the size of the embryo, and there are only three possible sizes: dwarf, normal, and giant. Furthermore, the dwarf and giant embryos can only be produced by experimentation, which alters the normal processes.

[1]The authors wish to express their indebtedness to Dr. Wm. H. Weston for his help and interest in this study.

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[4]Needham also states that maintenance energy has not been isolated and, therefore, little is known of the "metabolic cost of morphogenetic work." Although this may be true, one can nevertheless assume that the maintenance oxygen consumption is the same per unit mass regardless of the size of the embryo and that comparisons between different size embryos will instruct one as to the relative morphogenetic work.

Through the use of the slime mold *Dictyostelium discoideum* these two points can be satisfied because in this simple organism the phase involving morphogenesis is completely separated from the phase involving feeding, cell division, and growth, and in addition the sporulating individuals normally show a wide range in size. *D. discoideum*, Raper (1935), is a member of the Acrasiales, a group of slime molds characterized by the fact that its members are composed of aggregates of uninucleate amoebae (or myxamoebae). The life cycle of *D. discoideum* is subdivided into four stages: in the *vegetative stage* the myxamoebae grow and multiply, each acting independently. They feed on bacteria in the usual phagocytic fashion, and when they appear to have exhausted the food supply the *aggregation stage* sets in. From this point on, all processes of growth (ingestion, mitosis, etc.) cease, and morphogenesis alone proceeds. In aggregation the amoebae covering a certain area all flow in toward a central point. The size of the final fruiting body depends on the number of myxamoebae in this area. This is normally very variable and a whole range of fruiting bodies of different sizes results. The amoebae tend to join together in strands, and the whole aggregation pattern looks like a series of streams running down a mountain. When all the myxamoebae have reached the center they have formed a bullet shaped pseudoplasmodium which crawls on the surface of the substratum like a slug. This constitutes the *migration stage*. The migrating pseudoplasmodium stops and rights itself, the anterior tip becoming dorsal, a process which brings the organism to the fourth and last stage, the *culmination stage*. Differentiation has already set in, and the spore mass rises on a delicate stalk into the air. The mature sorocarp possesses a small basal disc, a slender, tapering, stiff-walled stalk (which is made of vacuolated amoebae compacted in a "cellulose" sheath), and an apical spore mass or sorus (a spherical mass of spores each of which represents an encapsulated amoeba). For a more detailed description of the life cycle of this organism see Raper (1935, 1937, 1939, 1940a, 1940b, 1941), Bonner (1944).

The culmination stage is of concern in the present study and, therefore, it would be well to discuss it further by giving a brief résumé of the previous descriptive work (Bonner, 1944). In the beginning of this stage, before any rising occurs, the vacuolated stalk cells are laid down in the center of the pseudoplasmodium, all of them encased in

a smooth, cylindrical, stiff-walled stalk sheath (Figure 1A). The spore cells have already partially differentiated at this stage, and soon afterwards they become completely encapsulated (Figure 1B). The rise is accompanied by two active internal movements: (*a*) the elongation of the stalk, and (*b*) the passing of the cells from the upper region, lateral to the stalk, onto the top of the stalk proper. As the culmination nears its end, the supply of these pre-stalk cells lying outside the stalk becomes depleted and the increase in height ceases (Figure 1C).

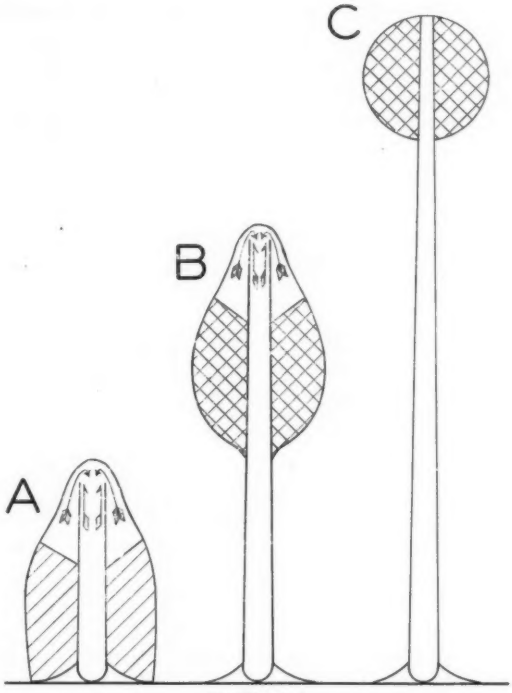

FIGURE 1

DIAGRAM ILLUSTRATING THE PROCESS OF CULMINATION

A shows the beginning of the process where the spore mass has not yet separated from the substratum. The arrows indicate the active movements involved in the process of rising, the central pair represents the extending of the stalk wall, and the distal pair represents the migration of cells to the top of the stalk. The diagonal lines show the presence of pre-spore cells. *B* is the middle of culmination where the pre-spore cells have become true spore cells as indicated by the cross hatching. *C* is the mature sorocarp where culmination, and therefore all movement, has ceased.

As may be seen from the preceding brief description of its cycle of development, Dictyostelium has the very qualifications that are essential, if work such as that of Tyler is to be extended beyond the limitations imposed by such material as sea urchin embryos. In this organism the processes of growth and accompanying ingestion and mitotic activity are normally separated from morphogenesis. Morphogenesis, fruiting, will only occur if the food supply has been depleted; in fact, fruiting is stimulated by starvation. Therefore, any observations made on the organism beyond the vegetative stage are on morphogenesis alone. Also, there normally occurs a great variation in the size of the organism. Because there is no growth in the later stage of development, the size of the sorocarp is entirely dependent on the number of amoebae that aggregated into one pseudoplasmodium. Despite this variation in size, each sorocarp is of approximately similar proportions, as will be shown later.

Since Dictyostelium is so preëminently suitable for morphogenetic study, the writers undertook an experimental investigation of the factors involved in rate of movement in sorocarp formation, and the results are presented in the following pages.

Methods

The organism was grown in a two-membered culture with the bacterium *Escherichia coli* on 1.5 per cent agar containing 0.1 per cent Bacto-peptone (method of Raper, 1939). The conditions were kept constant so that observations from different cultures would be comparable: the pH was kept between 6.5 and 7.0 which, according to Raper (1939), is well within the range of normal development conditions; the temperature was maintained at $16.5 \pm 5^{\circ}$ C; and the atmosphere surrounding the organism was always kept saturated.

The experiment consisted of photographing a number of culminating sorocarps at hourly intervals (for method of photographing see Bonner, 1944), and from these photographs the following measurements were made: the diameter of the sorus and the total height of the sorocarp at each interval; from these measurements the rate of increase in height was calculated. The latter was done by obtaining the hourly increase, discarding the measurements at the beginning and end which might not be indicative of a full hour's activity, and averaging the rest.

RESULTS

To understand the process of culmination more thoroughly the first step was to examine the shape of the curve obtained when the height of the individual fruiting body is plotted against time. This was found to be of a characteristic shape for all fruiting bodies, irrespective of their size (Figure 2).

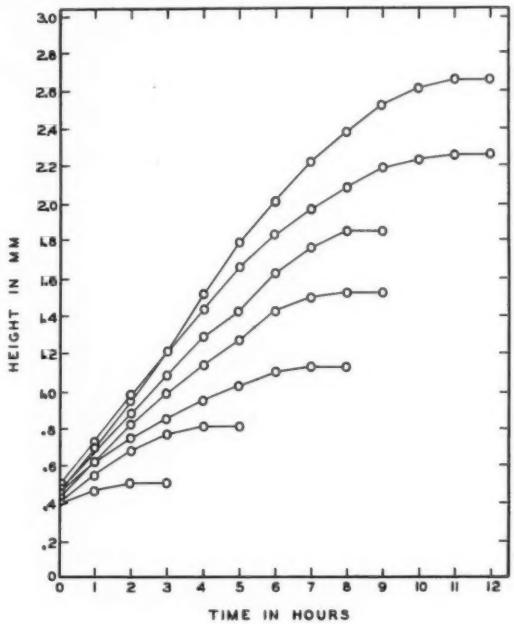

FIGURE 2

GRAPH SHOWING THE RELATION BETWEEN HEIGHT AND TIME IN SEVEN DIFFERENT SIZE SOROCARPS

Note that the characteristics of the curves are essentially similar for all sizes. [The complete increase in height curve for the smallest sorocarps is not recorded here because, for the sake of illustration, each curve was started at approximately the same heights (.40-.45mm.)].

The individual developing sorocarp may be described by three measurements: the height of the mature stalk, and the radius of the mature sorus, both of which are size factors, and thirdly, the rate

of increase in height. If an attempt is made to examine the relationship between these measurements it is found that there is a definite relationship between any two of the variables. This is plotted in Figure 3 in such a manner that, at the risk of slight distortion, these three relationships may be observed simultaneously. They may be expressed: $v = f(r) = f(h)$, where v is the velocity of height increase, r the radius of the mature sorus, and h the height of the mature stalk. These relationships are not necessarily linear. In fact, approximately linear curves can be obtained also by plotting these variables semi-logarithmically or as reciprocals. The scatter of these plots makes it difficult to establish a simple relation between v, r, and h, but they indicate that the rate of morphogenetic movement v, the radius of the sorus r, and the height of the stalk h are approximately proportional, and an increase in one will accompany an increase in the other two.

Discussion

The radius of the sorus r and the height h are an index of the size of the organism. Since r and h are linear dimensions, and since r is approximately proportional to h, one can conclude that all sorocarps are approximately proportional.

Since $r = f(h)$ one could use either variant as a measure of size to discuss its relation with the rate of increase in height v. But r is of more theoretical significance, because it is a direct index of the sorus weight that must be lifted into the air by the stalk.

First it should be noted that these results agree with those of Tyler, namely, that the smaller the organism the slower the rate of development.

The interpretation of the nature of the immediate causative agent of this relationship is a problem that deserves some attention. It is perhaps helpful to keep in mind that the rate of development can be expressed in terms of power, that is, the larger the organism the greater the power output per unit mass of protoplasm.

There is little or no evidence to indicate that two similar organisms of different sizes should have different types of protoplasm, one more powerful than the other. It is more plausible to suggest that the power output per unit mass of protoplasm is the same, and this is borne out by Tyler's observation

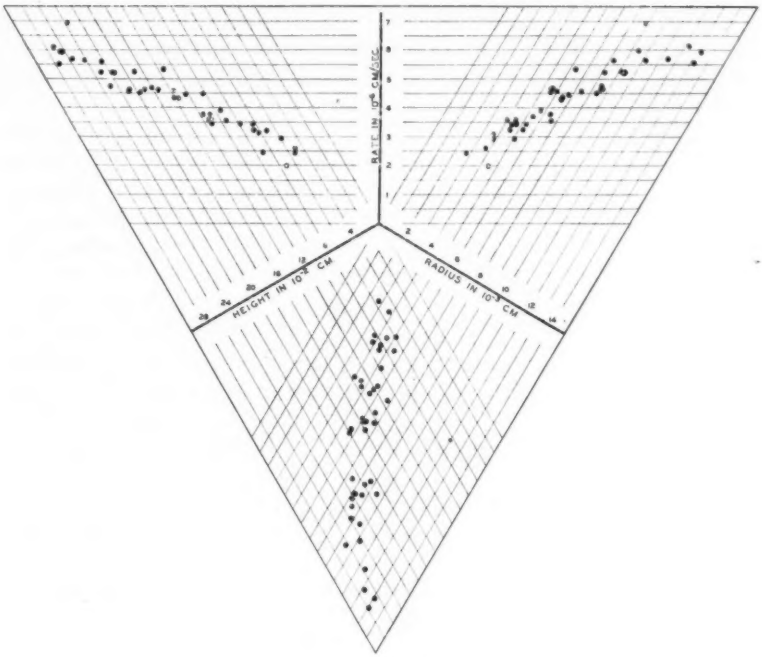

FIGURE 3

Three graphs plotted simultaneously showing: (1) the relationship between height of the mature sorocarp h and the radius of the mature spore mass r, (2) the relationship between h and the rate of increase in height of the sorocarp during culmination v, (3) the relationship between v and r. The advantage of this type of graph is that the interrelations of three variables can be easily observed, but the disadvantage is that the distribution of the plots is slightly distorted and their scatter tends to appear less than it actually is.

in the sea urchin that the oxygen consumption is the same per unit mass of protoplasm. Therefore it is equally plausible to suggest that there is an opposing force, an inhibition, that in some way is related to size, so that it becomes greater the smaller the organism. The hypothesis, then, is that there is both a morphogenetic force of the internal protoplasm that is equal for a unit mass in all members of one species under a given set of environmental conditions, and an opposing force that becomes greater the smaller the organism.

Tyler (1942) suggested that this opposing force in the case of the sea urchin may be the result of the action of the principle of similitude, a principle which states that if an object increases in size, retaining its linear proportions, the increase in surface area will be greater because it is a function of the square of the linear dimensions, and the increase in volume will be even greater because it is a function of the cube of the linear dimensions.[5] In the case of a sphere its surface increases as the square of the radius, and its volume increases as the cube of the radius. Therefore, as the size decreases there is less volume or mass proportional to the amount of surface area.

If the resistance to change in shape of the surface is constant irrespective of total size, then the smaller the organism, the more work has to be performed by the internal protoplasm to alter the surface shape. In the sea urchin Tyler (1942) points out the known fact that the blastula wall at the time of gastrulation is the same thickness for dwarf, normal, and giant embryos. Therefore, the resistance of this wall to invagination is the same regardless of size, but in the dwarf embryos more work has to be performed by a unit mass of protoplasm than in the larger embryos. Since, presumably, the protoplasmic instantaneous rate of performing this work is constant, regardless of the load (as demonstrated by the constant values for the oxygen consumption), it can only perform this additional work by taking a longer time.

It is more difficult to ascribe a physical entity to resistance to change in shape in Dictyostelium. A plausible possibility is suggested by an analogy to drops of rain on a window pane. It is everyone's common experience to see large rain drops roll down the glass faster than small ones, and see some even so small they do not roll down at all. The downward force is that of gravity which is a function of the mass or the volume, but this force is resisted by the cohesion between the glass and the water, which is a function of the surface area of water touching the glass. It is evident that the principle of similitude acts here, for in the larger drops their volume per surface ratio is greater, and therefore the downward force of gravity is greater.

In Dictyostelium the principle force is not the downward pull of gravity, but an upward morphogenetic force of some yet unknown

[5]See Thompson (1942, Chapter II) for a complete description of this principle.

character.[6] The morphogenetic force is probably internal for, if it were a surface force (such as surface tension), according to Laplace's law the total pressure resulting from this force would be inversely proportional to the radius of curvature.[7] That is, the smaller the sorus, the greater is the total pressure. Since larger sorocarps culminate faster than small ones, presumably the motivating force is not superficial but must be internal.

If the morphogenetic force is internal, assume that it is proportional to the mass or volume. Now the force resisting the upward movement could be the cohesion between the sorus and the stiff-walled stalk, which is proportional to the surface area of the stalk. Since the stalk and the sorus are always approximately proportional, the greater the size of the organism, the greater the volume per surface ratio, and therefore the greater the rate of rise, which is actually the case.

The cause for the rate of increase in height tapering off as culmination nears its end (illustrated in Figure 2) is not given by the above explanation, but when the nature of the interior morphogenetic force is known, a cause may become obvious.

In conclusion a word should be added concerning the importance of these considerations. Ultimately, most developmental physiologists seek the causes of developmental processes and patterns. In this paper a causative agent has been suggested (but not proved) for an unusual relation between the rate of development and the size of the developing organism. But little evidence has been obtained concerning the more fundamental problem of how the organism converts its potential energy into morphogenetic work. The little evidence obtained on this problem is that the surface of the developing organism is probably not a source of energy (because the more surface in proportion to the volume of the organism, the slower the rate of development) and therefore the source of morphogenetic energy is internal. It will, no doubt, be of some interest and value to establish definitely the causative element of the inhibition of the rate of development for

[6] It should be noted that the effect of gravity is too little in these small organisms to oppose the morphogenetic forces. This is evidenced by the fact that the effect of gravity is greatest in the large sorocarps yet they are the ones to rise most rapidly.

[7] $P = T\left(\frac{1}{R_1} + \frac{1}{R_2}\right)$

where P is the pressure, T is the surface tension, and R_1, R_2 the radii of curvature.

small organisms described in this paper. But it is of far greater interest and value to isolate and understand the method by which energy is made available for morphogenetic movement, and so far the only guiding information for this problem is that the source of energy must be internal.

Summary

The slime mold *Dictyostelium discoideum* is a suitable organism for morphogenetic study because, in its life cycle, growth occurs first, ceases, and is followed by differentiation alone. In the final phase of differentiation (culmination) the spore mass is lifted into the air, supported by a slender stalk. Since there is no food assimilation or mitotic activity during this process, it is truly a morphogenetic movement. The rate of increase in height of a number of fruiting bodies of different sizes was recorded during culmination by time interval photographs. An approximately proportional relationship was found between the rate of morphogenetic movement and the radius of the spore mass. From this relationship the following hypotheses were induced: (*a*) that the motivating force of morphogenetic movement is internal and not a surface force, and (*b*) that the spore masses of smaller fruiting bodies rise into the air more slowly than larger ones because the amount of cohesion between the spore mass and the stalk is greater in proportion to the internal morphogenetic force, by virtue of the action of the principle of similitude.

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GRADUATION OF HUMAN GROWTH CURVES

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A. INTRODUCTION

Barach (1945) has painstakingly assembled and computed "adequate" curves for growth in height and weight of human males and females. His final curves, however, are not "smooth," being subject to somewhat erratic fluctuations. These uncontrolled variations are more noticeable if one considers first and second differences. Such erratic behavior is found almost universally in actual population data as collected. Much of statistical and actuarial theory and practice has been developed to obtain suitably smoothed results to replace the crude collected data. The advantages of such smoothings are obvious and have been pointed out by many authors.

The smoothing of observed population statistics has two aspects of interest: first, the method of obtaining the smoothed result; second, the smoothed result itself. The purposes of this paper are, first, to develop methods for dealing with the Barach data; second, to present the result of applying these methods to Barach's data on the growth in height and weight of humans. The methods used are an extension of those developed in previous publications (Baker, 1943, 1944; Baker, Mead, and Regan, 1945).

B. DEVELOPMENT METHODS

In previous work with the growth curves of many biological entities it has been found that a formula of the form

$$\frac{y_{t+1}}{y_t} = 1 + \frac{a(t)}{b(t)} \tag{1}$$

as developed and applied by Carver (1924) for frequency distributions represents satisfactorily the first differences of the growth curves. This curve of first differences determines the growth curve except for

a constant of proportionality, k, and the beginning ordinate o. In this study k and o have been determined in order that the sum of the squares of the differences between the observed and smoothed values may be as small as possible. This principle of least squares is easy to apply in this case.

For other growth curves it has been sufficient to take

$$a(t) = a - t, \tag{2}$$

where a is the time of most rapid growth and

$$b(t) = b_0 + b_1 t + b_2 t^2, \tag{3}$$

where some of the b's may be zero. For human growth $a(t)$ and $b(t)$ must be modified. After considerable trial computation it was found sufficient, for the present range of data, to take

$$a(t) = a_0 + a_1 t + a_2 t^2 + a_3 t^3 + a_4 t^4 - t^5 \tag{4}$$

and

$$b(t) = b_0 + b_1 t. \tag{5}$$

If data going back to the time of conception were available, it might be necessary to modify our functions of t still further.

C. Application to Human Data

If we assume $t = 0$ at age 2, then we find the following expressions for proportional sets of first differences:

(6) Female weight in pounds

$$\frac{y_{t+1}}{y_t} = \frac{239{,}010.478 + 8{,}612.245t + 10.128t^2 - 453.924t^3 + 39.052t^4 - t^5}{256{,}593.574 - 8{,}771.091t}$$

(7) Female height in inches

$$\frac{y_{t+1}}{y_t} = \frac{15{,}440{,}799.71 + 248{,}246.41t - 79{,}232.36t^2 - 104.59t^3 + 2.31t^4 + t^5}{17{,}722{,}163.63 - 632{,}043.99t}$$

(8) Male weight in pounds

$$\frac{y_{t+1}}{y_t} = \frac{317{,}192.781 + 91{,}828.798t + 22{,}492.248t^2 - 835.646t^3 - 44.560t^4 - t^5}{295{,}513.863 + 158{,}025.192t}$$

(9) Male height in inches

$$\frac{y_{t+1}}{y_t} = \frac{9{,}310{,}342.0 - 154{,}918.3t + 541{,}409.7t^2 - 34{,}151.4t^3 + 63.0t^4 + t^5}{9{,}204{,}128.4 + 1{,}861{,}438.0t}$$

Proper factors of proportionality and beginning ordinates were then determined in each case by the method of least squares. Table 1 presents final results of this smoothing. If graphs are made of the observed and smoothed size values, it will be seen that the erratic ele-

TABLE 1

OBSERVED (BARACH, 1945) AND SMOOTHED GROWTH FOR HEIGHT AND WEIGHT OF HUMAN MALES AND FEMALES

	Female				Male			
	Weight (pounds)		Height (inches)		Weight (pounds)		Height (inches)	
Age	Observed	Smoothed	Observed	Smoothed	Observed	Smoothed	Observed	Smoothed
2	25.5	25.42	33.3	33.40	26.2	25.70	33.4	32.94
3	29.9	29.84	36.4	36.47	31.1	30.38	36.4	36.26
4	33.9	33.97	39.0	39.15	35.0	35.40	39.4	39.63
5	37.4	38.08	41.4	41.60	38.4	40.16	42.0	42.55
6	42.8	42.44	44.2	43.93	43.2	44.71	44.6	45.02
7	47.5	47.27	46.8	46.20	49.5	49.25	47.0	47.16
8	53.9	52.80	49.0	48.46	54.5	54.04	49.1	49.10
9	60.2	59.23	50.7	50.75	60.5	59.35	51.2	50.98
10	67.0	66.65	52.5	53.06	66.6	65.46	53.0	52.88
11	74.0	75.06	55.0	55.36	73.5	72.67	55.1	54.86
12	82.3	84.23	57.2	57.61	81.0	81.20	57.1	56.96
13	94.0	93.71	59.7	59.71	90.0	91.16	59.1	59.17
14	105.0	102.84	61.9	61.56	102.0	102.34	61.6	61.43
15	111.5	110.92	63.2	63.04	113.9	114.00	64.0	63.62
16	117.0	117.34	64.2	64.08	126.0	124.86	65.8	65.56
17	121.6	121.82	64.6	64.68	133.0	133.33	66.8	67.07
18	124.0	124.44	64.9	64.94	138.0	138.29	67.7	68.03
19	126.0	125.62	65.0	64.99	140.0	140.00	68.5	68.48
20	126.0	125.93	65.0	64.99	140.0	140.09	68.5	68.61

ments in the observed data have been removed in a reasonable and pleasing manner.

D. SUMMARY

Methods that are the extension of formulas previously found useful have been developed for graduating human growth curves without breaking such curves into arbitrary pieces as so many authors have done. These methods have been applied with good results to Barach's (1945) data on the growth in height and weight of human males and females.

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RELATIVE GROWTH IN RACES OF MICE PRODUCED BY SELECTION

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Phenomena of relative growth have been studied as ontogenetic processes (heterauxesis), or/and by comparing the sizes a part has attained in mature organisms (allomorphosis) of different body sizes and belonging to a fossil series, related contemporary living species, breeds, etc. The long history of the actual differentiation into the species or breeds to be compared is in any case usually hidden in the remote past, and the nature and causes of the evolutionary divergence are necessarily more or less obscure and problematical. Inferences and uncertainty about genetic relationships may be largely avoided by choice of favorable stocks of recent and known origin.

A. MATERIALS

The laboratory house mice here compared belong to a small (S_8) race about the size of Chinese waltzers, and a large (L_8) race, much exceeding ordinary mice in size, as judged from the standards of Gates, Fortuyn, and others (Grüneberg, 1943). These two races were produced from one and the same foundation stock by a few years (eight generations) of pedigreed selections, guided by progeny tests of 60-day weights (MacArthur, 1944), all mice sharing throughout the same environment, care, and feed (except that each sibship was fed by its own mother).

B. METHODS

The data are based on measurements of body and appendages of 784 mice, representing the two races, both sexes, and a range of ages from two to 456 days. For each specimen were recorded the pedigree, age, sex, total weight in grams, and four linear measurements in mm., taken by Sumner's (1909, 1915) methods: body length (BL),

snout to anus; tail length (TL), anus to fleshy tip of tail; ear length (EL), from apex to lowest notch of pinna; and foot length (FL), including the claw of the longest, middle toe of the left hind foot. Three indices were calculated, showing the ratios of tail, ear, and foot length to body length.

Means for each group alike in race, sex, age, etc., were used for plotting points on graphs. The data were finally analyzed by the relative growth equation, $y = bx^k$, x representing the body length, y the length of an appendage, k the constant of differential growth, and b the index of initial size. Values of b and k were determined by methods suggested by Feldstein and Hersh (1935), and the standard error of k ($S.E._k$), following Simpson and Roe (1939).

C. Differences between the Races

1. *Linear Measurements*

The divergence in total weight in the selected mice is accompanied by the expected marked changes in the linear measurements of the body and of the various appendages. That all these latter changes are real and significant is illustrated for 60-day-old males in the upper half of Table 1. The contrasts are as evident in the females.

TABLE 1

Comparison of selection races of house mice (males) in body weight, body length, absolute and relative linear measurements of appendages at 60 days of age; and in number of young per litter born to females (whose measurements are similar but smaller).

	Small race (S_8)	Large race (L_{38})	Difference Standard Errors
60-day weight (gms.)	16.14±.25	33.71±.31	44.1
Absolute length (mm.) of:			
Body (BL)	81.28±.44	96.73±.31	28.6
Tail (TL)	75.78±.58	85.54±.46	13.2
Ear (EL)	12.60±.07	13.82±.05	15.2
Foot (FL)	17.12±.10	19.05±.06	16.1
Relative length of appendage:			
TL/BL	0.931±.005	0.882±.005	7.0
EL/BL	0.159±.001	0.149±.001	7.1
FL/BL	0.211±.001	0.197±.001	10.0
Number of young per litter:			
First litter	5.00±.19	9.22±.70	4.6

2. *Degree and Direction of Allometric Changes*

The small race differs from the large, however, not only in a slower growth, smaller mature weight, and linear measurements, but also—and again entirely without our striving to alter them in any way—in having relatively longer appendages (tail, ears, and feet), and fewer young born per litter (lower half of Table 1). These features remain characteristic of the small race through life. The difference in each instance is much too great to be attributed to sampling errors, and too widespread and consistent to result from chance fixation of characters.

It will be noted that the races differ more significantly in weight, the sole criterion of selection, than in the associated linear measurements; more in body length than in appendage lengths; and more in absolute than in relative lengths of the appendages. Correlation coefficients, if determined, would doubtless decline in the same order.

D. Sex Differences

In both size races the 60-day females are significantly smaller than males in body weight and length. The appendages may differ little between the sexes in absolute length, but the indices show females to have significantly longer tails, ears, and feet (Table 2). These differences decrease again at 4 to 9 months of age, as the females approximate males in body length. In fact it proves futile to describe the sex differences in relative lengths of the appendages either within or

TABLE 2

Males and Females of the Small Race Compared at 60 Days in Body Weights, Linear Mesurements, and in Relative Lengths of the Tail, Ear, and Foot

	Males	Females	Difference Standard Error
Body weight (gms.)	16.14±.25	13.80±.19	7.5
Absolute length of:			
Body	81.28±.44	76.50±.63	6.2
Tail	75.78±.58	74.90±.48	1.2
Ear	12.60±.07	12.76±.06	1.7
Foot	17.12±.10	16.86±.05	1.4
Relative length			
TL/BL	.937±.005	.980±.004	6.1
EL/BL	.159±.001	.167±.001	6.0
FL/BL	.211±.001	.220±.001	8.4

between races, unless their dependence upon body length (and age) is recognized.

The differences in the three indices between races and sexes at 60 days are shown graphically in Figure 1, where each vertical line repre-

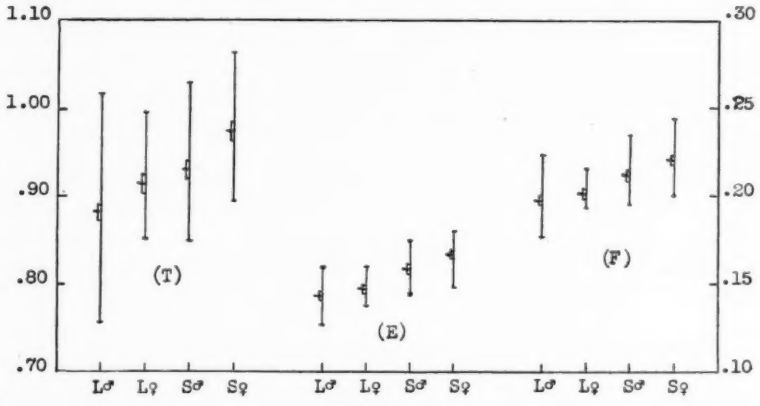

FIGURE 1

Graphic estimation of significance of differences between races and sexes in the ratios: (*T*), tail length/body length, to left hand scale: (*E*), ear length/body length, and (*F*), foot length/body length, to scale at right.

sents the extreme range of observed variation, and the short horizontal line protruding to the left, the mean. The small rectangle extends vertically to a distance three standard errors above and below the mean. When the rectangles do not overlap vertically, the means are considered significantly different. This method was adopted from Simpson and Roe (1939), following Lee and Dice.

E. Heterauxetic Changes

Data tabulated to this point have emphasized allomorphic differences existing at 60 days of age between adults of the two strains and sexes. Relative growth of the appendages was then studied as dynamic heterauxetic processes during the growth of the mice in the postnatal period up to 18 months of age.

The important fact was at once revealed that each appendage, comparatively small and short when first measured after birth, increases

steadily to a maximum relative length when the body is 40-75 mm. long, and thereafter again becomes relatively shorter until the definitive body length is reached (Table 3).

TABLE 3

CHANGES IN RATIOS, APPENDAGE LENGTH TO BODY LENGTH, DURING POSTNATAL BODY GROWTH FROM 28 TO 120 MM. LONG

	Shortly after birth	At peak	At greatest body size
$\frac{\text{Tail length}}{\text{Body length}}$	.46	.96	.77
$\frac{\text{Ear length}}{\text{Body length}}$	.09	.18	.12
$\frac{\text{Foot length}}{\text{Body length}}$	.21	.28	.16

The general growth is slow in the small race, the curve having flattened at about 85 mm. body length; in the large race growth is faster, ceasing at about 120 mm. The interracial size differences are already evident at birth, and increase progressively at 30 days, 60 days, and full maturity. The appendages, at first comparatively short in the fetal and newborn mouse, lengthen faster than the body itself (the brief tachy-heterauxesis phase) until a maximum is attained. Then appendage growth slows down abruptly, and falls definitely behind that of the body (the prolonged brady-heterauxesis phase). As a result, when mice of a corresponding age are compared, in the very young the appendages are relatively longer in the large race, but in mature and old mice the appendages become, and then remain, relatively shorter in the large race.

The sex differences are similarly accounted for. Males grow faster than females, and go more rapidly through all the relative growth changes in size of appendages. During their brief phase of rapid growth, the appendages are relatively larger in males, and the peak and decline reached sooner in the males. Thus at 60 days and later the males have advanced farther in the brady-heterauxesis phase.

Averages of the measurements of various age groups of both races and of both sexes were plotted on a double logarithmic graph to make them more amenable to analysis from the point of view of relative growth. In Figure 2 the mean lengths of the various appendages are

plotted against the various body lengths. For each set of points the values of the four components of the allometric equation were calculated. From these equations regression lines were drawn on the graph. They fit the plotted points rather well. Table 4 summarizes the calculated values of b and k, and the standard errors of k.

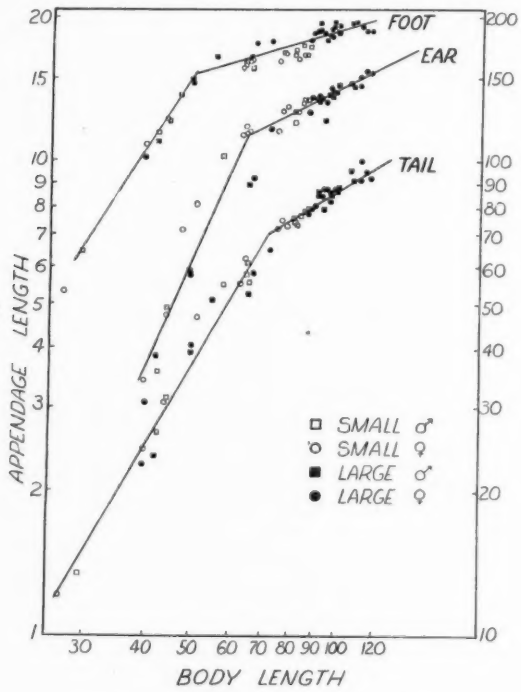

FIGURE 2

Double log graph of regression of appendage length on body length, irrespective of age. F for foot, and E for ear length to scale at left. T for tail length to scale at right. Each curve includes four sets of points.

Several notable features stand out from a study of the points, curves, and equations. (*a*) The large and small races and both sexes follow the same pattern and course in allometric development. The curve representing relative growth in each race and sex coincides

TABLE 4

VALUES OF THE ALLOMETRIC CONSTANTS, b AND k, FITTING THE GROWTH DATA IN FIGURE 2

		b	k	SE_k
Tail	1st phase	.035	1.782	.110
	2nd phase	4.02	.668	.043
Ear	1st phase	.0007	2.324	.128
	2nd phase	1.43	.497	.052
Foot	1st phase	.033	1.567	.030
	2nd phase	3.61	.357	.040

with the growth curve of the other race or sex. The large race curve might be considered an extrapolation of the curve for the small race. (*b*) Each appendage studied has two distinct phases of development, the first exhibiting positive heterogony, the second, negative heterogony. In every instance the value of k, the coefficient of heterauxesis, is well above one in the first phase, and much less than one in the second phase. (*c*) The break between the two phases occurs earliest for foot growth (at a body length of about 45 mm.), then for ear growth (when the body is about 65 mm. long), and latest for tail growth (at approximately 70 mm. body length). The breaks are thus not synchronous. No special efforts were made to define the inflection points more precisely.

The absolute length of appendages varies with race, sex, age, and body length, but when size (body length) is taken out by being held constant, the variations in appendage length become small. That is, at any corresponding body length the two races have about the same size appendages in either sex, in general regardless of age. All the mice collectively form one homogeneous allometric population or tribe, with b and k values identical, or nearly so, in the two races. The size changes effected by selection were accompanied by no marked changes in these rate determinants.

The curves for ontogenetic relative growth of the appendages coincide well in the large and small races, take the same course up to the break and for some distance beyond it. The large race thus recapitulates the development of the small, and passing beyond it, exhibits a further reduction and foetalization of the appendages.

Runts. Distinctly different is the allometry situation with the

"runt" individuals, phenotypically resembling pituitary dwarfs, which segregate clearly, like Mendelian recessives, from some small-race pairings. Their general growth is noticeably retarded at 10-12 days, body length seldom reaching 55 mm. When compared with their normal sibs at this size their body and appendages show significantly different proportions specifically localized down the body axis, the snout being blunt, the ears relatively short (index .145 vs. .180 for normals), the feet rather short (index .255 vs. .291), and the tail relatively long (index .921 vs. .880).

F. Discussion

Emphasis was first laid on the considerable differences existing between the relative dimensions of the parts of the two selected races. The subsequent reduction and analysis of data yield an entirely new and different picture of development in these races. For each appendage studied the two races are shown to follow the same curve, and hence have the same values for the components of the equation of relative growth. In other words, the group curves describing the allomorphosis found in the two selected races are identical with the ontogenetic curves describing the heterauxesis typical of each race. To our knowledge this study represents the first instance of such an identity in mammals based on a complete comparison of phylogenetic or group curves supplemented by ontogenetic ones. This identity, in spite of the apparent differences of the relative dimensions of adults, is the result or mode of expression of a common and persistent genetic rate determinant, unmodified by eight generations of severe selection for size. Fundamentally it indicates that the value of k, the equilibrium or differential-growth constant, is dependent upon processes which have a great measure of genetic stability. Similar genetic stability was reported for fowls by Lerner (1943), a selected strain retaining the k value of the parent stock.

The plus and minus selections having been directed only at changing body size as a whole (total weight at 60 days), the mouse races presumably differ genetically wholly or mainly in size or growth rate factors exerting a measurable phenotypic effect by or before that age. Since the selections took no note of body proportions, size of any part, length of appendages, or number of young in a litter, the races could hardly differ materially in specific factors controlling appendage

size or litter size, unless, of course, there were some chance fixation or elimination of such genes, or fresh mutations during the selections. Nor would the races be expected to differ in relative growth constants, such as k or b.

The variations in lengths of tail, ear, and foot are thus part of a quantitative complex dependent upon (a) those general size and growth rate factors (multiple factors, etc.) regulating body size as a whole, acting with (b) whatever associated basic factors fix and determine the b and k values for each appendage. Since the rate determinants did not change, there is no indication that specific, independent, local-acting factors are involved in the divergence of the races.

Varying relative size of a part in any micro- or macro-evolutionary series depends upon the general size factors and the inherited rate determinants for the regions and parts involved. The simplest allometric system in taxonomic groups of common ancestry is that where size polygenes change, but b and k values are single and unchanged. Small and large races or species would then be "proportionate"; they would be strictly proportionate, only in the rare event of the k value being exactly one. In our small race, as probably in most so-called "proportionate dwarfs" as ateliotics, proportions are altered by k values less or more than one. Marked disproportionality may result from either an abrupt break of k into two components, or from distinct mutative change in either constant, b or k, whether single or interrupted by a break. Change with striking localized, major or pleiotropic, effects on particular parts, characterizes many simple Mendelian mutant forms, such as runt, short ear, or short tail in mice, chondrodystrophy in man, etc.

Marked changes in rate determinants controlling relative growth of appendages more or less independently of, or superposed on, body size factors occur frequently in speciation processes. *Mus bactrianus* of central and east Asia, closely allied to the house mouse, resembles our small race in size, but differs sharply in allometric constitution, the tail being "relatively shorter," containing fewer vertebrae and rings (Fortuyn, 1929-30). In the wild deer mouse, *Peromyscus maniculatus,* the subspecies *bairdii* has a distinguishing short tail. Proportions differ greatly among the *Peromyscus* species, and Clark (1941) concluded that "body size and proportions cannot be attributed

primarily to general size factors and heterogonic growth," variations in tail, body and foot lengths being "due primarily to special size factors, genetic and developmental." In taxonomic divergence of long standing the allometric changes in corresponding characters may be enormous. Castle and Reed (1936), for instance, noted that "other things being equal, the larger the rabbit, the larger proportionately are the ears, its skull, and its legs." "The genetic agencies which produce large body size automatically produce long ears, and vice versa"; for most appendages, the allometry relations in rabbits are practically the opposite of those found in the house mouse.

The approximate recapitulation of phylogenetic or group curves by ontogenetic curves has been demonstrated in the relative growth studies of horses (Robb, 1935), of anthropoid apes (Lumer, 1939), and of dogs (Lumer, 1940); it has also been supposed to be true for titanotheres (Hersh, 1934). Lumer (1940) sets forth the hypothesis that the converse of such recapitulation is also true, namely, that if the adults of different groups (species, varieties, etc.) are represented by points along a single curve, then the ontogenetic curves are the same in all the types under consideration. But all published accounts of the identity of group curves with ontogenetic curves have dealt with organisms the relative growth rate of whose parts was represented in each instance by a single value of k. The present investigation offers a somewhat different and novel aspect in that the curve for each set of measurements has two distinct values of k. In spite of this fact the identity of the ontogenetic curves with the group curves still obtains. While all adults presumably are represented by points within the second phase of the curves in question, the first phases also follow single curves for each set of measurements. That this should be so in this study is not astonishing, since the mice used in the experiment were not of very remote relationship. However, for groups long separated with an unlike hereditary background, it would not seem safe to predict the behavior of individuals in the first phase from the observed and calculated relations existing in the second phase. Therefore Lumer's hypothesis concerning the recapitulation of ontogeny by phylogeny should be accepted with reservation until critical examples of its general application are found.

The fact that development of parts in mice occurs in two demonstrable phases has been shown previously with different methods by

Green and Fekete (1933), and by Clark (1940). The cause of the break in each curve, however, is still a subject for speculation. In the selected mice the breaks for the tail and ear curves occur at a body length of about 65-70 mm; L_8 individuals attained that size about 22 days after birth, S_8 individuals about 30 days after birth. For the foot curve, the break occurs when mice have attained a length of about 45 mm., i.e. when L_8 mice were 9-13 days old, and S_8 mice close to 20 days old.

Some authors have suggested that the onset of puberty is, like a metamorphosis, a physiological phenomenon of sufficient importance to produce such an effect as a break in the curves. The breaks in the tail and ear curves do coincide roughly with the attainment of maturity. Green and Fekete (1933) state that the second straight line for limb measurements coincides at its beginning with the appearance of the centers of ossification. In all curves then age operates per se with very little effect, if at all, in the determination of the points of break in the value of k. Absolute size, on the other hand, is certainly closely correlated with the incidence of the second phase of growth. Whether this correlation is due to the action of size as an immediate or remote cause, or to the dependence of size upon some third common causal agent, or to an interaction of these various relations cannot be determined from our data. It is quite possible that glandular conditions (e.g., of the pituitary) play a prominent rôle in the determination of both the onset of puberty and the incidence of a change in the value of k; but, even then, the question as to which is the cause, which effect, size or the glands, remains unsolved.

In this connection it is interesting to extrapolate our results into the future when a small race may conceivably be produced which never attains a length of 70 mm. If this point of selection should ever be attained and if absolute size is the sole modifier of this second phase plus the attainment of sexual maturity, then sterile mice would be produced and further selection balked. This condition would be, in effect at least, similar to that in the runt mice or those affected with pituitary dwarfism (Snell, 1929).

The several parallels between the results produced in this laboratory experiment by artificial selection and those found in nature, presumably due to natural selection, may interest ecologists. So-called "climate rules" have been formulated from time to time (Rensch,

1936, 1939) to describe the tendency for body size, appendage size, or litter size to be distributed with relation to temperature clines. Thus Bergman's Rule states that, in exposed homothermal animals, races of larger body size tend to inhabit the colder, more polar or alpine, parts of the species range. Allen's Rule notes that races of cold climates often have relatively short ears, feet, and tails. Rensch's Rule shows that larger litters oftenest characterize the cold climate races. These three rules, discovered many decades apart, are quite certainly not independent, but intimately related through a mutual dependence upon general size factors. In this study body, appendage, and litter sizes form a correlated quantitative complex, partly under common genetic control, and responding together to selection aimed at changing body size.

G. Summary and Conclusions

Selections for body weight in the house mouse produced small and large races which differ significantly in linear measurements, in relative length of the appendages (tail, ear, hind foot), and in litter size.

For each appendage there proved to be two phases of relative growth, the first of tachy-heterauxesis, the second of brady-heterauxesis. The breaks between phases occur when the body is 40 to 70 mm. long, but are not synchronous for the three appendages.

The difference in relative length of appendages between races, sexes, and ages are shown to depend primarily upon body size and the genetic factors controlling it.

Length of each appendage is controlled by general body size factors, acting with or on characteristic specific rate determinants for heterauxesis. These determinants were not changed by the body size selections. The mice all form one allometric tribe, not differing in b or k constants.

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THE RATE OF GROWTH OF TADPOLES IN VARYING CONCENTRATIONS OF RINGER'S SOLUTION

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Studies on the influence of varying concentrations of salt solutions on regeneration of both fresh water and marine organisms have been reported repeatedly but to our knowledge no studies of the rate of growth in intact organisms under such conditions are available. Goldforb ('07) found maximal regeneration in marine hydroids when sea water was diluted 15-20 per cent. Other studies by Lloyd ('14) and Keil ('32) on other marine organisms are consonant with these results. Sayles ('34) found maximum regeneration of tadpole tails in tap water and a decrease in regenerative activity with increasing concentrations. There has been little attempt at the analysis of the possible factors operating in these cases. Keil ('32), however, regarded the effect as not solely osmotic but involving specific salt effects.

The present study attempts to determine the influence of various Ringer's solutions on the rate of growth in tadpoles and to analyze the contributions of some of the factors involved in the results. Al-

[1]The results reported in this paper are the products of a series of investigations on the factors in tadpole growth carried on by students working under the direction of the senior author at the American Museum of Natural History. The investigation was originated as a teaching project in which each "honors" student contributed by working on one phase of a common problem. One of the topics selected originally, yielded what seemed to be information of sufficient significance to be followed up analytically by successive "generations" of students. Some of the earlier results were published in the student publication as given in the bibliography of the present paper. The principal experiment described here, however, was not previously published and was carried through by the junior author. The other experiments are given here only in summary form and only in so far as they contribute to the analysis of the particular problem. The students whose reports have been drawn upon in the present analysis are: Lt. Murray Davidson, A.A.F.; Pfc. Walter Sencer, U.S.A.; Pfc. Robert Soberman, U.S.A.; Pfc. Wilbert Staub, U.S.A.; Cpl. Gordon Kaplan, U.S.A.; Lt. Paul Metzger, A.A.F.; and of course the junior author's. We wish to thank Dr. Frank Beach, Director of the laboratories of Animal Behavior, American Museum of Natural History, for so generously making the facilities of his laboratory available to us. We are indebted to the Research Committee of the College of the City of New York for grants-in-aid for this project.

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though the analysis cannot be considered as complete, it is felt desirable to present the more significant results and conclusions at this time particularly since the continuance of the project under present conditions is doubtful.

Methods

Tadpoles of *Rana pipiens* were secured by the usual implantation techniques (Rugh, '34). They were raised in dilute Ringer's (usually 1/10 or 1/4R.) until reaching a length of about 15 mm. at which size the animals have reached the definitive tadpole form and have been feeding for two or three days.

The animals were then selected for uniformity in size. When the proper number of animals had been picked they were distributed to the experimental dishes at random. Three animals were raised in each 250 cc. fingerbowl containing about 100 cc. of solution. When an animal died in a dish it was replaced by one of the animals in the last dish so that all dishes held three animals at practically all times except for the last dish of the series. In this dish the number of animals varied from time to time. In this way the factor of crowding, influential on tadpole growth (Adolph, '31), was kept at minimal variability.

The animals were fed oatmeal cooked in distilled water. Although this diet is known to be improved with liver (Davidson, '42) none was used here as good growth on a low salt diet is attained on oatmeal alone. Feeding was done daily, enough food being put into each dish to insure persistence until the next feeding, but great excess was avoided.

Animals were measured once a week in length only as this is the most convenient and reliable method (Schaper, '02). For this purpose the animals were put into a moist petri dish, placed over millimeter-ruled graph paper and the length was read by looking directly down at the tip of the head and tail. Such measurements were found to be reproducible to within one-half a millimeter.

The solutions were made up with single distilled water (Barnstead tin-lined still). The solutions used were as follows: Distilled water; 0.001 Ringer's (i.e., 1 milliliter Ringer's in 1 liter of solution); 0.005 R.; 0.02 R.; 0.25 R.; 0.40 R.; 0.50 R.; 0.75 R.; and 0.90 Ringer's. Tadpoles will survive for some time in full Ringer's but in

the present experiment none was used as survival and growth are too poor to provide significant growth curves. Twenty-four animals, in eight dishes, were set up in each concentration used.

Results and Analysis

The results are summarized in Table 1 and illustrated graphically in Figure 1. From these it can be seen that the animals grew best

TABLE 1

Concentration	No. of animals alive at end of experiment	Average size plus standard deviation at end of experiment in millimeters	Range in millimeters Minimum	Maximum
Distilled water	24	24.8- .38	22.0	28.5
.001 Ringer's solution	24	30.8- .54	25.0	35.5
.005 Ringer's solution	24	34.8- .67	29.0	40.5
.02 Ringer's solution	23	40.1- .65	33.5	45.5
.25 Ringer's solution	20	46.0-1.34	36.5	58.0
.40 Ringer's solution	22	43.0-1.36	34.5	56.5
.50 Ringer's solution	20	37.5- .87	30.0	45.0
.75 Ringer's solution	10	38.1- .64	35.0	41.0
.90 Ringer's solution	10	29.3- .89	23.0	33.5

in 0.25 Ringer's solution and their growth fell away to almost 1/3 as great in distilled water on the one side and to about ½ in 0.90 Ringer's solution on the other. The presence of this optimum at 0.25 Ringer's was first discovered by Soberman ('42) who used only three different concentrations and distilled water. This optimum was subsequently verified in all investigations, seven different experiments in all. It was the purpose of the investigations which followed Soberman's discovery to analyze this phenomenon and to evaluate the contributions of the various factors in it.

Several factors suggest themselves as playing a rôle in the retardation of growth in concentrations other than 0.25 Ringer's. These may be considered under the headings of direct toxicity of distilled water, pH, water balance (osmotic effect), and salt balance. Of course the

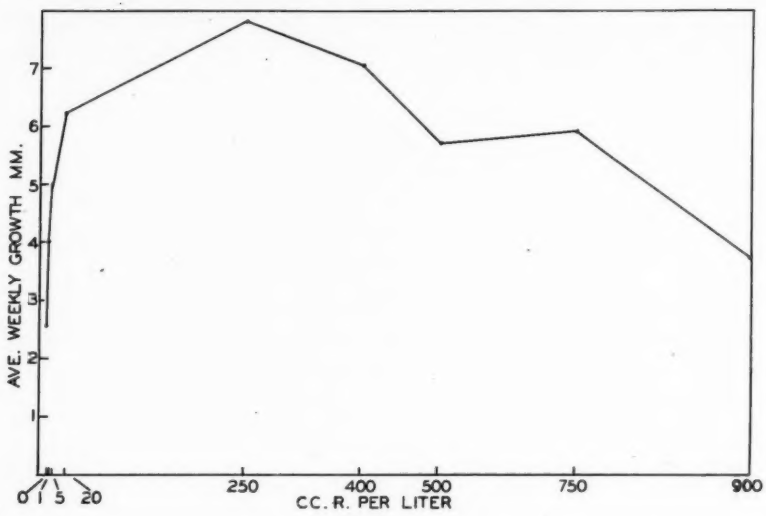

FIGURE 1

GROWTH OF TADPOLES AS INFLUENCED BY SALT CONTENT OF WATER
Ordinates—Average weekly growth of tadpoles.
Abscissae—Milliliters Ringer's solution per liter of final solution.

retardation on either side of the optimum might well be the result of different combinations of factors. Let us first consider the possible rôle of these factors in the inhibition of growth at concentrations below the optimum.

1. *Toxic Factor*

The presence of a toxic factor in distilled water derived from metal stills is a common experience particularly in working with marine organisms. However such toxicity cannot play any rôle in the present experiment since the entire fluid content of every solution is derived from the same distilled water. Soberman compared the various Ringer's solutions he used with tap water and found that O.25 R. animals grew about as well as those in tap water. That this rate of growth is favorable when compared with tadpoles in natural ponds is shown by the fact that our tadpoles grew better and metamorphosed ahead of those in natural ponds in this region. Of course the

temperature difference makes exact comparison impossible, but the favorable showing indicates that the growth of tadpoles in our laboratory conditions is more favorable than that in natural conditions generally. At any rate, if there were any toxic factor present it does not seem plausible that the addition of the small quantities of pure salts involved in the lower concentrations would serve to detoxify it. Yet growth was greatly improved by small quantities of salts as will be discussed below.

2. *pH Factor*

The pH of the solutions was taken when prepared and again after the animals had lived in it for a few days. La Motte indicators were used. The pH tended to drop with dilution and to drop slightly during use. The recorded variation was from 5.4 for 0.001 Ringer's after two days to a high of 7.9 for freshly prepared 0.90 Ringer's. An experiment was set up to test directly the importance of these pH variations. Three groups of 24 tadpoles each were set up in 0.25 Ringer's. In one group, the pH of the 0.25 Ringer's solution was adjusted to about 5.4 with HCl, in another to about 8.0 with NaOH, and one group was left unmodified at the pH of 7.4. Though these pH values moved downward two or three tenths of a point while animals lived in the solutions, the relative values remained fairly constant. By way of comparison, control groups were run in 0.001 Ringer's at the unmodified pH of 5.4 and in 0.75 Ringer's at the unmodified pH of 7.8. The results showed the usual decrease in growth in these unmodified groups as compared with unmodified 0.25 Ringer's solution. The group in the acidified 0.25 Ringer's solution showed practically identical growth with those in the unmodified 0.25 Ringer's solution and much better growth than those in 0.001 Ringer's solution at the same pH. The group in 0.25 Ringer's solution with the pH raised to 8.0 showed a slight decrease in growth. This decrease, however, amounted to only 1/3 of the difference between the unmodified ¼ Ringer's and ¾ Ringer's solutions. It may be concluded, therefore, that pH played no rôle in solutions more dilute than 0.25 Ringer's solution and was only a small factor in the decrease in growth in solutions above 0.25 Ringer's solution.

3. *Water Balance (Osmotic Pressure) Factor*

On a priori grounds it might be expected that the osmotic differential between distilled water and the internal osmotic pressure of the tadpole might be so great as to result in an excess intake of water, particularly as Adolph ('25) has found that the intake of water in tadpoles is proportional to the O.P. differential. It might be supposed that by putting an excessive burden upon the regulatory mechanisms, the water level of the tissues might be disturbed or the work involved in water excretion raised, so as to interfere with growth. A number of experimental approaches were taken for the evaluation of this factor. The percentage of water in tadpoles raised in distilled water and in 0.25 Ringer's solution was compared (Soberman '42), but no difference was found. There is also no apparent edema or other sign of failure of the regulatory mechanism. A study of kidney size was undertaken by Staub ('44). He raised tadpoles in various concentrations of Ringer's solution and when they reached 40 millimeters in length he fixed them in Bouin's fluid. The Bouin's was washed out in alcohol and the kidneys carefully dissected out. These were transferred to water and weighed quickly after a standardized blotting treatment to remove excess water. It was found that the kidneys of tadpoles raised in distilled water were more than twice as heavy as those raised in a 0.25 Ringer's solution. The weights showed a trend downward in higher concentrations, but the data were not extensive enough to establish statistical significance for the differences in the other categories. The clear-cut increase in kidney size, however, in distilled water animals indicates that there is indeed an increased activity of this organ. It does not follow, however, that this increase is responsible in any important measure for the retardation of growth. There are, in fact, many indications that the O.P. differential is not of major significance.

One such indication is seen in the data of Table 1 and Figure 1. Here it can be seen that the rate of growth is not at all proportionate to the change in O.P. differential. If we assume that the O.P. of tadpole tissue is approximately that of full Ringer's (Backman and Runström, '12) it can be seen that the tendency of water to enter the animal would be decreased by only 0.1 of 1 per cent in .001 Ringer's solution as compared with distilled water. Yet the rate of growth is markedly improved by this minute addition of salts. Similarly the

principal improvement in growth has been accomplished by the addition of only 20 cc. Ringer's solution per liter even though the drop in osmotic differential tending to drive water into the animal has decreased only two per cent. It does not seem possible, therefore, to ascribe the decreased growth in any considerable part to the purely osmotic phenomena involved.

In another experiment (Kaplan, '42) it was found that if the tadpoles were transferred for one hour from distilled water in which they were being raised into a 0.50 Ringer's solution, a marked improvement in growth resulted. The procedure adopted was checked against any carry over of salts back into the distilled water. It would seem, therefore, that the animals can grow well in distilled water, provided they have access to a supply of salt.

Further indication that O.P. differential is not the dominant factor in the retardation of growth is seen in the fact that as mentioned above, in Soberman's initial experiment, the animals grew as well in tap water with its low osmotic pressure value as they did in the optimal 0.25 Ringer's solution.

Further evidence is the fact that when a feeding procedure was followed in which large quantities of lettuce were kept in the dishes at all times the growth of the tadpoles in distilled water was not appreciably less than in 0.25 Ringer's solution (Metzger, '44, unpublished; Davidson, '42). Although it is possible the excess of lettuce may have raised the osmotic pressure slightly, this effect could only have been very small even if all the salt were leeched out of the lettuce.

It can be seen from the above discussion that the same experimental results which indicate lack of effect of O.P. lend support to the idea that the limiting factor in growth of tadpoles in distilled water is the absence of appropriate salts. The strong positive effect of small quantities of salts, the effect of temporary immersion in salts, the effect of excess lettuce, and the growth in tap water may be cited in this connection. But these same results indicate that, in so far as this may be a salt effect, it is one which depends not on the total quantity of salt, but presumably upon one or more of the salts present in small concentrations of standard frog Ringer's solution. It is interesting in this connection to find that when tadpoles are removed from a favorable Ringer's solution and placed into distilled water, they do not lose appreciable chloride to the distilled water even after two or three

days (Kaplan, '42). This work was done on small tadpoles of Rana pipiens and also on large bullfrog tadpoles. Using a modified Mohr test for chlorides, it was found that for two to three hours after introducing a large tadpole into a small quantity of distilled water (following an appropriate series of washings through distilled water) traces of chlorides could be found, but even these disappeared from the solution after 24 hours. This remarkable capacity for holding chlorides is deserving of more extended study, but even in this preliminary form it suggests that the salt metabolism of the animal may well be maintained in distilled water as long as the minimal amount of salt is available to the organism from its food or water.

The problem of why tadpoles' growth falls on the other side of the optimum has not as yet been made the subject of any analytic experiments. It is perhaps worthwhile to point out some of the indications available from the present data.

Unlike the region below 0.25 Ringer's, the results in the higher concentrations are roughly in inverse proportion to the increase in concentration. The irregularity that appears in the principal experiment of the present paper at 0.40 Ringer's solution is exceptional. In the three other experiments in which the data are comparable a rather uniform drop in rate of growth is shown. Since a deficiency of salt is out of the question, it seems probable that the effect is due either to an inability to secure water or to a more direct toxicity of the high salt content of the environment. The percentage of water in tadpoles raised to the same size in various concentrations was determined (Soberman, '42). This failed to show any appreciable difference between animals raised in distilled water, 0.25 Ringer's solution, or 0.75 Ringer's solution. There would thus seem to be no marked breakdown of the regulatory mechanism in any of these concentrations. If, therefore, the water or salt balance is disturbed at the higher concentrations, the effect must be more subtle than a breakdown of regulatory mechanism.

Summary

Tadpoles were raised in various concentrations of Frog Ringer's solution. Optimal growth occurred in 0.25 Ringer's solution. At lower concentrations growth falls off, so that in distilled water it is about one-third as great. On the other side of the optimum, growth de-

creases until in 0.90 Ringer's solution it is about one-half that in 0.25 Ringer's solution. From an analysis of the data in this experiment and the consideration of a number of other experiments, it is concluded that the decreased growth in dilute solutions is probably due to the absence of enough of some critical salt rather than osmotic imbalance. In the higher concentrations, water balance disturbance may be of greater significance.

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PREPUBERTAL CASTRATION IN THE MALE CHIMPANZEE, WITH SOME EFFECTS OF REPLACEMENT THERAPY

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Of the many reports of the effects of prepubertal castration, few deal with primates and fewer still include any anthropological data. The following report represents the first such information in the chimpanzee.

Don, the son of Pan and Nana, was born at Orange Park, Florida, May 2, 1934, and there was nothing in his early life to differentiate him from any of our other infants. He was castrated by Dr. Harry A. Peyton of Jacksonville, Florida, at the age of 22 months. At that time both testes, both epididymides, and portions of both vasa deferentia were removed. During the period from June 23, 1937, to July 7, 1941, Don was a member of the New Haven colony, but the remainder of his life has been spent at the Yerkes Laboratories of Primate Biology, Orange Park, Florida. Like all of our animals he has been a subject in numerous psychological experiments. At present, Don is a young adult and except for the absence of testes and scrotum would not be distinguishable from a normal male of the same age. An intact male of this age would have practically attained his greatest height although he would continue to increase slowly in weight for some time (1).

Don has had three short periods of treatment with androgens and two with estrogens. In December, 1941, under nembutal anesthesia, two pellets containing 150 mg. testosterone propionate were embedded subcutaneously and while Don was under the influence of the drug, X-ray photographs and anthropometric measurements were made.

[1]The author wishes to thank the many who have aided with this investigation. Special thanks are due to Dr. R. M. Yerkes who instituted this study and to Dr. H. W. Nissen who took most of the X-rays and made most of the anthropometric measurements.

Subsequent tests by Nowlis and Young (2) revealed a marked increase in dominance. The persistence of this change in dominance led to the surgical removal of the fragments of the pellets after one month and to removal of the nodules of connective tissue in which they had been embedded a month later. Microscopic examination of the tissue removed revealed no trace of the pellets, however, no further X-rays or measurements were taken at that time.

In the fall of 1943, it was decided to repeat the dominance tests and to follow any changes under hormone therapy more systematically and over longer periods of time than had been done previously. The changes in dominance are reported separately (3). Before treatment with the sex hormone, Don was anesthetized and X-ray photographs and anthropometric measurements were taken. These were repeated one month after the last dose of male sex hormone, just before the second period of treatment with the same hormone and again after his 11th birthday. Don was given 50 mg. per day of methyl testosterone[2] from December 23, 1943, to January 26, 1944, and from October 12, 1944, to November 2, 1944. He also received 2.0 mg. per day of alpha estradiol from July 7, 1944, to August 24, 1944, and from December 7, 1944, to January 4, 1945.

Development of Sex Behavior

The development of sex behavior in this animal is perhaps best summarized by the quotation of notes[3] from his diary. Most of these excerpts could be duplicated in the files (4) of the other young male chimpanzees of our colony; there is little indication of any deviation of behavior from that of an intact animal. In fact, his development of interest in the opposite sex and the integration of the complete sexual act were more rapid and occurred at an earlier age than has been the case with several of our intact animals.

2 yrs., 8 mos. (McCulloch)—Masturbated with foot or by raking penis through cage wire while sucking observer's finger.

3 yrs., 8 mos. (Fletcher)—Ever since Don has been in the New Haven colony he has had erections when being petted. Further, he has shown a

[2]The methyl testosterone (Oreton-M) and alpha-estradiol (Progynon-DH) were generously supplied by the Schering Corporation, Bloomfield, N. J.

[3]The nonpertinent portions of these notes are omitted but they are otherwise unedited. The age of Don at the time of the observation was made and the name of the observer (in parentheses) are given before each item.

desire at the same time to have his penis in contact with the individual petting him. No new important qualitative aspects of such behavior have appeared, but during the last few weeks the above described behavior has been more striking than previously. When a person approaches the cage and pets him, he immediately shows great excitement, baring his teeth, etc. He has an erection and makes a strong endeavor to contact his penis with the person's arm, coat, or other part of the body. Such behavior will usually last as long as the petting is continued.

3 yrs., 9 mos. (Fletcher)—When anyone approaches his living cage he immediately has an erection and solicits attention and bodily contact.

5 yrs., 5 mos. (Nowlis)—Erections are very frequent, apparently related to expectancy of food, scratching or other favors, for obtaining the desired reward usually inhibits the erection immediately.

5 yrs., 8 mos. (Grether)—A characteristic pose of Don's when attempting to gain attention is to stand erect in front of the observer, with penis erect and very conspicuous.

6 yrs. (Grether)—Don and Helene were put together. There was a brief embracing, but no apparent signs of excitement or fear on the part of either. Don, with an erection, soon began grooming Helene's genitalia, in which she fully coöperated.

6 yrs., 4 mos. (McNeal)—After drinking milk at the evening feeding on Sept. 17, Don took his cup and darted quickly into his cage. He could not be induced to give it back, although he came forward for food or to be petted. He licked the cup, inspected it, chewed it, hugged it to his breast with both arms, and used it to masturbate with.

7 yrs., 8 mos. (Young)—The sexual behavior of Helene when she was observed with Dick and Don is characteristic of the adult females. Don, despite castration, has been observed to copulate, with complete intromission, with Helene and Gamma and once with Dina. Dina obviously had not initiated the action.

8 yrs., 11 mos. (Hebb)—Don was put with Gamma in M-2 today. He entered Gamma's cage, mounted, made three or four thrusts and broke away. A second attempt was made within a few minutes, with a very careful insertion by Don; this also was interrupted after three thrusts, this time by Don's reaching for food which was lying near.

This distractibility during copulation has not been noted in any of our other animals. Before the second series of hormone treatments repeated efforts were made to reproduce the observation with no success.

Copulatory Ability

It is unfortunate that more complete records were not made of the first tests. These consisted merely in placing Don with a suitable female for one hour and recording the number of copulations and the

presence or absence of signs of ejaculation. Later, more complete records were kept. In any such tests there are a number of factors which may cause considerable difficulty. For example, it is impossible to control masturbation. The younger males especially are often observed in this act and the usual presence of fecal material, with which their hands are often smeared, on their penises indicates that the practice is widespread. Failure of an animal to copulate may simply mean that he has recently masturbated and is unable to achieve an erection. There is also the difficulty of having a receptive female at the proper time. This was partially overcome by planning the hormone treatments so that the chosen female would be in maximum swelling when needed. Before any treatment with oral sex hormones was instituted, Don, in one test, copulated 10 times within one hour. Under the influence of methyl testosterone the maximum number of copulations was two per hour but usually one could find evidence of ejaculation after these. Under treatment with alpha-estradiol the maximum number of copulations noted was four in one hour which was also the largest number observed during the periods of rest between treatment. Only during the periods of treatment with methyl testosterone was any unmistakable evidence of ejaculation found, although at other times there was occasionally a very slight indication of glairy material in Don's meatus.

Growth of Genitalia

In Don's diary there is only one reference to the size of his penis. At the time the first X-rays were taken and before any treatment with sex hormones the notation was made that Don's penis was larger in size than those of intact males of the same age. This complete lack of retardation in growth of the penis, which has continued, contrasts markedly with the fact that the prostate was barely palpable before the first period of treatment with methyl testosterone, but, at the conclusion, rectal palpation revealed a well formed prostate. There was also a slight growth in the length of the erect penis during this period—from 13½ cm. to 14½ cm. The work of Tandler and Goss (5) on the Scopecs would indicate that a more or less infantile penis could be expected in a prepubertal human castrate so this penile growth represents possibly a species difference. However, what growth did occur in the penis of the Scopecs was in the corpus cavernosus

urethra and since this body forms a considerable portion of the conical penis of the chimpanzee the difference may lie in the anatomy of the organ and not in the effects of hormone deficiency.

Growth in Weight

The weights of a series of normal male and female chimpanzees and of Don are shown graphically in Figure 1. The curves for normal animals represent the medians of the weights of all the animals which have been under observation at this laboratory. No detailed

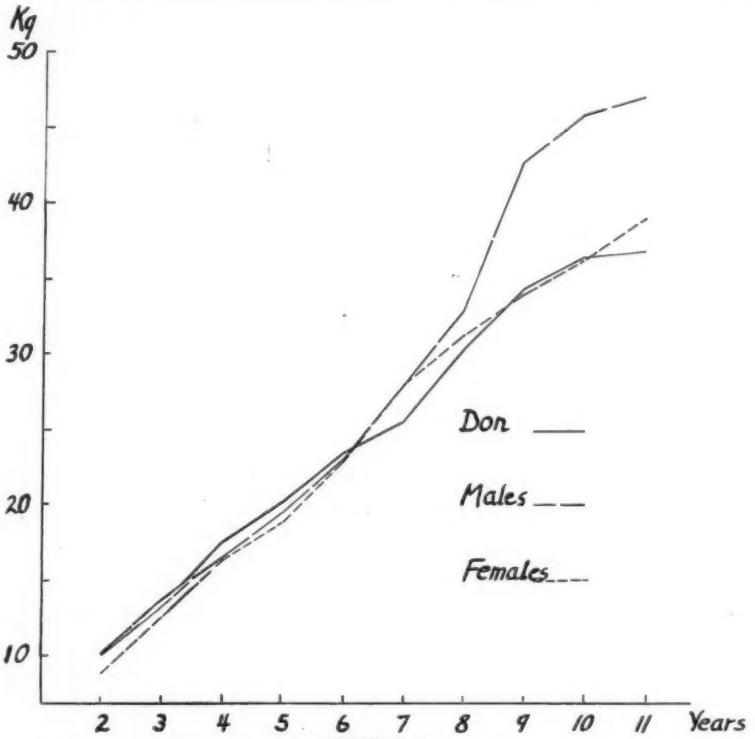

FIGURE 2

Medians of Weights of Male and Female Chimpanzee and the Weights of the Castrate Male—Don

report need be given as much of the data is already available (6) and the remainder is to be published elsewhere (1). The similarity between the weight curve for Don and for the females is extremely interesting, but some doubt as to the validity of any conclusion is occasioned by Figure 2, which represents the weights of three males

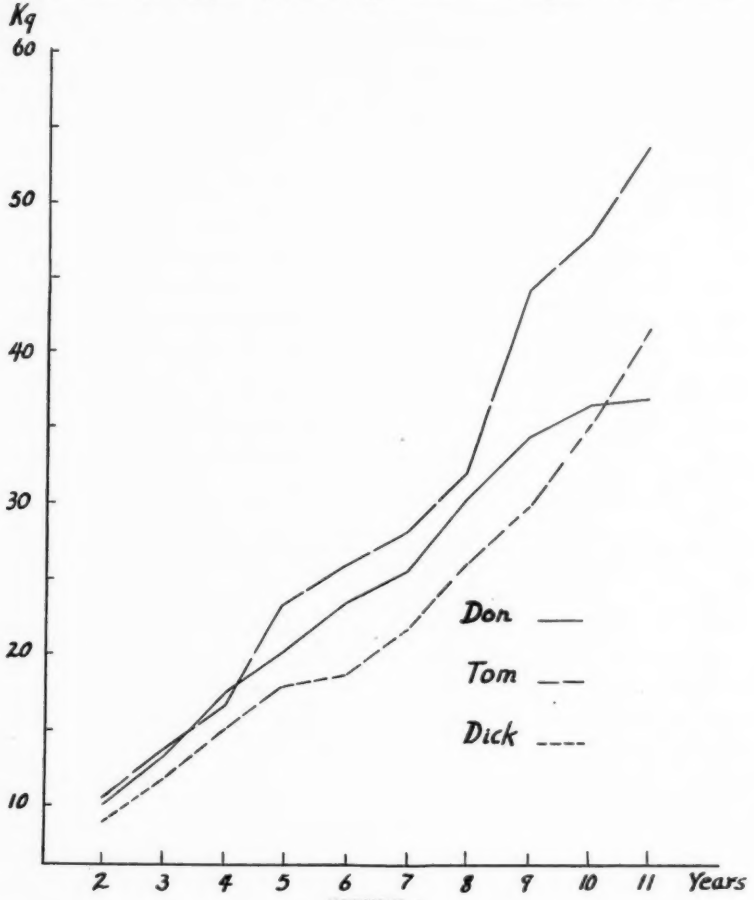

FIGURE 2
WEIGHTS OF TOM AND DICK (INTACT MALES) AND OF TOM (CASTRATE MALE)

all born at Orange Park. It will be seen that until the age of 11, Don's weight was consistently greater than that of Dick and less (except at age of 4) than the weight of Tom. It is important to remember that the diet and living conditions of these three animals have been practically identical and that they have often been caged together for long periods of time. It is apparent that there was a slowing down in growth rate on the part of Don after the age of nine years, but whether this represents a portion of the total picture of the results of castration must remain conjectural until further data is available.

Weight changes during the periods of hormone treatment were not consistent; their extent was within the limits of the other animals and their direction was variable.

Growth of Skeleton

There was considerable bone growth during the period over which X-rays were made but there was no indication of any spurts in rate. This was not unexpected in view of the report of Howard and Vest (7). There is not much difference between Don's final pictures (age 11) and those of two normal males at age 12. In all three animals the epiphyses are either closed or are apparently closing but, as might be expected from the disparity in ages, Don definitely has the lower bone age. Work in progress here (1) will amply supply control material within a few years and further comment will be made at that time.

It is commonly considered that in the primate prepubertal castrate there is an overgrowth of the long bones, but in our somewhat scanty material there is little or no indication of such an effect in Don. The pertinent measurements are collected in Tables 1 and 2. In Table 1 the name, age, and sex of the animal are given in the first three columns, then the ratio between the thigh length and sitting height expressed as a percentage, and in succeeding columns the knee length, sitting height; upper arm, sitting height; and the forearm, sitting height ratios (8). For convenience Don's ratios are placed between the males and females of each age group. In Table 2 a frequency distribution of the ratios is given with Don's ratios underlined.

Anyone who has made measurements of an active, wriggling animal can appreciate the fact that considerable error may occur unless extreme care is used. More exact figures are possible in the case of a

TABLE 1
LONG BONE-SITTING HEIGHT RATIOS
The starred animals represent unpublished material kindly supplied by Dr. A. Schultz.

Name	Age	Sex	Thigh L. Sitting H.	Knee L. Sitting H.	Upper A.L. Sitting H.	Forearm L. Sitting H.
Al	7 yrs. 0 mos.	M	40	30	36	35
Bill	7 2	M			44	
Pan	7 0	M			41	
Dayton*	7 6	M	39	32	38	33
?*	7 6	M	39	32	38	35
Don	7 7	Castrate	39	37	40	36
Bentia	7 6	F	39	42	39	38
Lita	7 0	F	38	38	35	33
Soda	6 6	F	39	41	37	36
Wendy	6 3	F			43	
Mamo	7 11	F	37	35	36	34
?*	9 1	M	40	32	38	35
?*	9?	M	38	31	38	36
?*	9 6	M	35	30	39	34
Don	9 7	Castrate		38	38	36
Dwina	9 0	F			45	
Bokar	20	M	38	38	40	37
Tom	12	M	40	40	41	38
Dick	12	M	39	39	43	39
Bill	11 8	M	33	38	35	32
Don	11	Castrate	38	39	38	37
Mimi	10 3	F	33	38	35	33
Nana	13	F	39	40		
Nira	10 3	F	34	35	37	34

dead animal or an animal under anesthesia. Some of the measurements supplied by Dr. A. Schultz were from dead animals and those of Don were taken while he was anesthetized, but it was thought best to give the ratios to only two places and to suggest that no significance should be attached to differences of less than two (corresponding to an estimated error of about 5 per cent). It is apparent from Table 1 and strikingly shown in Table 2 that there is little to suggest an overgrowth of the long bones in Don. The differences in the knee length-sitting height ratios in the 7-year- and 9-year males appears to be significant, but it should also be noted that this is not continued in the final age group nor is it apparent in the 7-year female group. Of greater significance is the more or less random distribution

TABLE 2
FREQUENCY DISTRIBUTIONS OF LONG BONE-SITTING HEIGHT RATIOS
In each case the ratio of the male castrate is in bold face.

Group	Thigh L. Sitting H.	Knee L. Sitting H.	Upper Arm Sitting H.	Forearm L. Sitting H.
	39	30	36	33
Males about 7	39	32	38	35
years old	39	32	38	35
	40	**37**	**40**	**36**
			41	
			44	
	37	35	35	33
Females about 7	38	**37**	36	34
years old	**39**	38	37	**36**
	39	41	39	36
	39	42	**40**	38
			43	
		30	**38**	34
Males about 9		31	38	35
years old		32	38	**36**
		38	39	36
	33	38	35	32
Males about 11 years	**38**	38	**38**	**37**
old or more	38	**39**	40	37
	39	39	41	38
	40	40	43	39
	33	35	35	33
Females about 11 years	34	38	37	34
old or more	**38**	**39**	**38**	**37**
	39	40		

of Don's ratios in Table 2. There is no indication in the tables of any systematic shifting of the ratios as puberty is passed, but a re-examination of these data will be made when the more exact figures on normal growth which are accumulating here become sufficient.

DISCUSSION AND COMPARISON WITH HUMAN DATA

It would seem that the effects of prepubertal castration in the human should be well established and indeed one may find long discussions in current physiologies, etc. Among the effects of prepubertal castration that are regularly listed are an infantile character of the penis, scrotum, and prostate and an overgrowth of the long bones as compared to short and irregular bones. The literature usually cited to support the concept of excessive growth in the long bones is not too convincing. Lipschütz (9),

for example, in discussing the study of Tandler and Goss (5) of that peculiar Roumanian and Russian sect called Scopecs, states that the penis and scrotum are infantile and the leg lengths excessive but he compares the leg lengths of these Scopecs with the average length of leg of Frenchmen! The most detailed study of human castrates is that of Wagenzeil (10), who studied a group of 31 eunuchs who had been attached to the household of the last Chinese Emperor. These individuals had been subjected to a very mutilating operation (removal of all the external genitalia) so that only information as to body form was available. They had been castrated at from 9-29 years of age, had been castrated from 28 to 60 years, and ranged from 45 to 75 years of age at the time of examination.

Several interesting comparisons may be made from the data collected by Wagenzeil (10). There were 10 of these individuals castrated at the age of 15 or less and 11 castrated at the age of 21 or more. A comparison of the leg lengths (rather symphysis heights) in these two groups gives a "*t*" value of 3.49 for the difference which is significant at less than the 1 per cent level. If one compares the total heights less symphysis heights, the "*t*" value is 0.0293, indicating no significant difference between the two groups. Thus it would seem to appear that the trunk heights of prepubertal castrates and of postpubertal castrates are very similar but that the leg lengths and thus total heights of the prepubertal castrates are greater. This interpretation of the data was given in a recent review (Gardner and Pfeiffer, 12), but in view of the fact that eight of the prepubertal and eight of the postpubertal castrates as determined above are listed by Wagenzeil (10) as being "humped-back," one may well question Gardner's and Pfeiffer's conclusion, especially since the degree of the kyphoses as shown by the photographs of the eunuchs was quite variable. If one compares the ratio between the body lengths and symphysis heights of the non-humped back castrates, there are eight in the postpubertal group ranging from castration ages of 18-29 and only two in the prepubertal group with castration ages of 14 and 15. The ratio for the 14-year castrate (.902) lies within the range of ratios of the postpubertal group (Range .867-1.007 average .958), all of whom were castrated after the age of 18 years, while that of the 15-year castrate (.851) lies slightly beyond the range. It is of interest that the height of the 15-year castrate is only 1.4 cm. less

than that of the 19-year castrate with the smallest ratio, while their symphysis heights are the same. Since in both cases the deviations of the prepubertal ratios from the average of the postpubertal ratios are in the same direction (greater leg length as compared to body height) this may possibly be significant. At least it may represent a trend and thus supports the contention of Gardner and Pfeiffer (12) mentioned previously. In total height one of these prepubertal castrates lies considerably above the average and another considerably below. Clearly these are random results. Gardner and Pfeiffer (1943), in their discussion of these same data, did not include the 15-year castrate in their prepubertal group but rather in the postpubertal group. Under this condition the range in ratios of the postpubertals is .851-1.007 average .946 and the remaining prepubertal castrate has a ratio of .902, well within the range of the postpubertal castrates.

Depending, then, on one's belief as to the age at which a castration is no longer prepubertal, one might either conclude that there is perhaps a relative and a significant absolute increase in leg length in these prepubertal castrates or that there was an absolute increase in leg length with no indication that there was also an increase in relative leg lengths.

The evidence that any difference in the two groups is due to the castration is not unequivocal. Since these individuals entered the emperor's household shortly after the operation (13), it follows that the early castrates may have had a much more adequate diet during their years of rapid growth and that, if measurements had been made before the kyphoses became prominent, there might have been little relative differences between the two groups except that the prepubertal castrates would have been larger in all dimensions.

Heller, Nelson, and Roth (14) use these data of Wagenzeil's to support their hypothesis that in the tall eunuchoid there is a minimal secretion of androgen which is sufficient in amount to cause bone growth without being sufficient to induce sexual maturation while in the short eunuchoid no androgen is secreted. They compare the heights of the 11 eunuchs castrated before the age of 17 with the heights of 250 Chinese men as reported by the same author (11). Since most of these eunuchs came from a small region in Hopei Province (13), the possibility of their being a more homogeneous group than a miscellaneous collection of 250 Chinese men, should have been con-

sidered. As has been shown above, the evidence from the group of castrates is directly opposed to the theory of Heller, Nelson, and Roth. There is the further fact that since Wagenzeil states that 9 of the 11 had varying degrees of kyphosis, any conclusions which would be influenced by factors *decreasing* the total height are not valid. If the kyphoses had not been present, these prepubertal castrates might have been considerably taller than the postpubertal castrates and possibly many would have exceeded the normal values for the region from which they came, yet, as shown above, this might not have been necessarily the result of the castration.

We can, then, call into question the concept that one result of Prepubertal castration in higher primates is an overgrowth of the long bones. The evidence from the human is not unequivocal and there is no evidence for the condition in our prepubertal castrate chimpanzee. Is it not possible that such a claim of overgrowth of the long bones would never have been made if this condition were not such a prominent symptom of human eunuchoidism? Despite the long and tedious nature of the work, further studies on the effects of castration in the higher primates seems indicated.

The tendency for the weight of Don to approach that of females of the same age seems to need no further comment nor do the several effects of replacement therapy. Each period and in fact the total period of treatment was probably too short to have any marked effect except on sexual ability and behavior.

Conclusions

1. The weight curve of a prepubertally castrate chimpanzee tends to approach that of the female chimpanzee. This is especially true after the ninth year.
2. There is no overgrowth of the long bones in the prepubertally castrated male chimpanzee.
3. The growth of the penis in the prepubertally castrate chimpanzee equals that of the intact animal.
4. The development of sexual behavior in the prepubertally castrate chimpanzee is similar to that in the normal animal.
5. Copulation records under estrogenic therapy were similar with those in control periods.
6. Short periods of treatment with androgens has no effect upon

the weight of the prepubertally castrate chimpanzee but ejaculation occurs and there is a decrease in the number of copulations per hour.

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TOLERANCE OF LARVAE OF DROSOPHILA FOR AMINO ACIDS: TYROSINE, PHENYLALANINE, AND ALANINE*

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For the series of investigations of which this report is a part, the larvae of Drosophila melanogaster are used as test animals. Known concentrations of amino acids are added to a basal dietary medium, prepared as described by Wilson and Birch (1944). Variations in definite growth patterns between the test and control insects are recorded in order to determine the concentration to which the animal is tolerant as well as to observe modifications of the pattern which may develop as a result of ingestion of the amino acid.

It is of value to make an analysis of the effects produced by related amino acids in a single report. This paper therefore includes data obtained by addition of various concentrations of the three amino acids, l-tyrosine, dl-phenylalanine, and dl-alanine to a sterile basal medium containing 7.5 grams of fresh baker's yeast per 100 ml. of culture medium. It is thus possible to emphasize and compare both the differences and similarities which may exist. Experiments were performed at each of the following concentrations for each of the amino acids: M/10, M/25, M/50, M/250, M/500, M/1000, M/2500, and M/5000.

Phenylalanine, of these three, is considered an indispensible amino acid for rats and some other vertebrates (Rose, 1938). Little is known concerning the requirements of invertebrates other than for the case of certain microorganisms, but, in general, these latter seem to require all three amino acids (Hegsted, 1944; Hutchings and Peterson, 1943; Kline, 1943). In the chick, tyrosine may replace phenylalanine (Almquist and Grau, 1944) and in species of *Streptococcus* and

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Lactobacillus, dl-alanine may replace the required pyridoxine (Gunsalus and Bellamy, 1944; Snell, 1945).

A few facts are known concerning the rôles of these amino acids in metabolism. Tyrosine is an integral part of many enzymes, hormones, and immune proteins of vertebrates and thus plays an essential rôle in regulation of growth processes. Its ingestion accelerates the deposit of glycogen in the liver of rats (Butts, Sinnhuber, and Dunn, 1941) probably because it is readily converted into glycogen (Mitchell, 1942). Both tyrosine and phenylalanine stimulate the deposit of keto acids in the rat liver (Waelsch and Miller, 1942). Tyrosine also causes an increase of glucuronic acid excretion in the rat (Martin and Stenzel, 1944). Its adequate ingestion prevents hyperplastic thyroids (Pal and Bose, 1943) and as a part of the same picture, tyrosine acts as a lipotropic agent in rats (Channon, Mills, and Platt, 1943). Earlier, Hoffman and Gudernatsch (1933) showed its relationship to thyroid activity by presenting evidence that tyrosine stimulates metamorphosis of tadpoles, although alanine and phenylalanine seemed to be more effective differentiators. That phenylalanine as well as tyrosine also accelerates intracellular metabolism was demonstrated by Miller and Reimann (1943) for developing ova of rabbits.

Hammett (1936) analyzed the influence of these three compounds on developmental growth of *Obelia*. He concluded that alanine, phenylalanine, and tyrosine are alike in that they cause increased metabolic activity. While l-tyrosine is a participant in some process concerned with differentiation, phenylalanine as a precursor of tyrosine is of secondary importance in differentiation. He considers d-alanine to be a "general tissue 'baustein.' "

Results and Discussion

1. *Tyrosine*

Even in saturated solutions tyrosine is not toxic when ingested by developing Drosophila larvae. A concentration of M/5000 or lower does not significantly alter any process of growth but all concentrations from M/2500 to saturation (M/250) cause an acceleration in the rate of moulting from first to second instars (Table 1). At the higher concentrations, the acceleration is maintained throughout larval life so that rate of pupation is also slightly accelerated in concentrations from M/1000 to M/250. The percentage columns in

TABLE 1

AVERAGE LENGTH AND WIDTH IN MILLIMETERS OF LARVAE TREATED WITH TYROSINE: AGE IN HOURS FROM MEDIAN HOUR OF OVIPOSITION

		Control				M/250 (sat.)				M/2500			
Age	Instar	No.	%	Length	Width	No.	%	Length	Width	No.	%	Length	Width
48	1	139	49	1.57	.234	94	36	1.56	.246	82	29	1.57	.232
	2	142	51	1.98	.262	167	64	1.95	.267	199	71	2.02	.276
74	2	186	57	3.04	.444	79	34	3.07	.448	137	41	3.10	.459
	3	141	43	3.69	.535	156	66	3.48	.539	200	59	3.68	.530
96	3	231	100	5.09	.736	189	100	4.92	.738	266	100	5.08	.735
120	3	169	84	4.89	.767	68	65	4.94	.768	109	98	4.99	.799
	P	32	16			37	35			2	2		
		Control				M/500				M/1000			
48	1	56	34	1.56	.245	67	26	1.55	.230	67	29	1.57	.245
	2	109	66	2.05	.295	188	74	2.05	.293	161	71	2.05	.240
72	2	88	49	3.06	.462	85	42	3.17	.480	71	40	3.12	.484
	3	93	51	3.82	.541	116	58	3.87	.551	106	60	3.68	.530
96	3	102	100	5.18	.813	131	100	5.22	.831	118	100	5.44	.832
120	3	55	42	4.88	.792	60	40	4.90	.747	24	23	5.26	.847
	P	76	58			88	60			81	77		

P—pupae.

the table illustrate this point. At every age where two stages of development occur the greater percentage of test animals is found in the later stage of development. Since this accelerated differentiation is accompanied by no significant difference in rate of growth in width or length, it seems possible that tyrosine stimulates the moulting hormone or enters into the structure of that hormone just as it does into the structure of thyroxine and other organic catalysts of vertebrates.

Tryptophane (Wilson, 1945), proline (Wilson, 1941) and arginine (Wilson and Birch, 1943) have produced a similar acceleration of the rate of larval moulting. Tyrosine behaves like tryptophane and proline in that it does not produce an accompanying increase in length or width as does arginine. It may be that arginine expedites moulting by increasing potential or available energy rather than by entering into the hormone-reacting system. Data to be presented later in this report show that alanine behaves in somewhat the same manner as arginine. Moulting however involves such complex reactions that speculation on the rôle of tyrosine or any of these acids is pointless without more knowledge of the entire moulting process. These results do seem to make it possible to state that tyrosine augments some reaction concerned with the expression of differentiation in Drosophila.

Highly saturated solutions in which crystals of tyrosine are thickly suspended throughout the media do inhibit moulting to a slight extent. In Table 2 the percentage columns show fewer test animals at the later stage of development at any given age. This effect is in no way

TABLE 2

AVERAGE LENGTH AND WIDTH IN MILLIMETERS OF LARVAE TREATED WITH SATURATED SOLUTIONS OF TYROSINE: AGE IN HOURS FROM MEDIAN HOUR OF OVIPOSITION

		Control				Saturated			
Age	Instar	No.	%	Length	Width	No.	%	Length	Width
48	1	5	5	1.57	.216	35	14	1.56	.231
	2	95	95	1.90	.269	217	86	1.97	.287
72	2	124	40	2.96	.416	118	43	3.04	.456
	3	182	60	3.65	.518	155	57	3.66	.526
96	3	158	100	5.16	.752	168	100	5.06	.760
120	3	74	64	4.85	.736	190	94	4.95	.745
	P	42	36			12	6		

P—pupae.

toxic to the larvae and the flies emerge at the same rate as the experimental imagoes and seem to be as fertile and viable.

There is a slight increase in width of test larvae over control larvae at the saturated concentrations. Of 23 chances for comparison, the length of the test animals in 11 cases was significantly greater than the controls, in four cases less and in eight cases equal. When width is considered there are 14 cases where the width was greater in the rest, six where the width was less, and two where it was equal. If the increased width is interpreted to mean a larger fat body (Wilson and Birch, 1944), then a high concentration of ingested tyrosine may be deposited as glycogen in the cells of the fat body as it is deposited in the liver of vertebrates (Butts, Sinnhuber, and Dunn, 1941). On the other hand, tyrosine may act to increase the deposit of fat in the fat body just as it catalyzes the removal of fat from the livers to fat depots in the rat (Channon, Mills, and Platt, 1943).

These experiments do not present conclusive evidence for increased metabolic rate such as was produced when tadpoles (Hoffman and Gudernatsch, 1933), rabbit eggs (Miller and Reimann, 1943), and *Obelia* (Hammett, 1936) were treated with tyrosine. Drosophila larvae express increased metabolism by a decreased rate of growth in length and width and by a prolonged larval period.[1] Although averages from several or from individual experiments failed to show any significant difference in length between tests and controls it is possible to show that the test animals were smaller by comparing individual larvae from a series of experiments. Larvae from three experiments in which M/500 tyrosine was used were compared and 76 per cent of the test larvae were smaller than their controls. Three hundred forty-six such pairs at 48 and 72 hours of age were studied. Since the rate of pupation was also increased at this concentration, tyrosine seems to accelerate metabolism as well as differentiation.

2. *Phenylalanine*

Rate of moulting is accelerated by phenylalanine but only at higher concentrations and to a smaller extent than for tyrosine. Table 3 shows that it occurs at the first moult but does not occur at the second when larvae are exposed to M/1000 phenylalanine. At the concen-

[1]Dorothy Thornton, unpublished results on effect of administration of dinitrophenol.

TABLE 3

AVERAGE LENGTH AND WIDTH IN MILLIMETERS OF LARVAE TREATED WITH PHENYLALANINE: AGE IN HOURS FROM MEDIAN HOUR OF OVIPOSITION

		Control				M/100				M/250			
Age	Instar	No.	%	Length	Width	No.	%	Length	Width	No.	%	Length	Width
48	1	41	30	1.56	.233	49	39	1.50	.232	60	41	1.53	.233
	2	95	70	1.90	.262	75	61	1.86	.262	86	59	1.90	.265
72	2	85	92	2.86	.426	113	93	2.91	.437	150	98	2.74	.413
	3	7	8	3.72	.512	8	7	3.85	.543	3	2	3.83	.550
96	2	2	1	2.70		2	1	3.03	.450	1	1	3.07	
	3	115	99	4.58	.603	113	99	4.56	.639	131	99	4.53	.624
120	3	67	94	4.99	.772	72	96	4.94	.769	81	96	5.01	.753
	P	4	6			3	4			3	4		
		Control				M/500				M/1000			
48	1	97	78	1.57	.240	53	67	1.57	.250	84	65	1.54	.239
	2	27	22	1.89	.273	26	33	1.89	.284	46	35	2.00	.249
72	2	98	91	2.83	.412	66	88	2.83	.420	103	94	2.83	.414
	3	10	9	3.87	.531	9	12	3.84	.528	7	6	3.95	.512
96	3	105	100	4.51	.617	41	100	4.52	.611	89	100	4.51	.632
120	3	129	98	5.03	.734	15	100	4.95	.755	111	96	4.92	.740
	P	3	2							3	4		

P—pupae

tration of M/500 both moults are accelerated but there is no acceleration of rate of pupation, nor is there any effect on length and width. Concentrations below M/1000 are ineffective in modifying growth and at M/100 and higher retard growth in length and width and thus retard moulting and pupation. Table 4 shows the effect of treatment

TABLE 4

AVERAGE LENGTH AND WIDTH IN MILLIMETERS OF LARVAE TREATED WITH M/10 PHENYLALANINE: AGE IN HOURS FROM MEDIAN HOUR OF OVIPOSITION

		Control				M/10			
Age	Instar	No.	%	Length	Width	No.	%	Length	Width
48	1	24	36	1.54	.227	50	91	1.46	.226
	2	42	64	1.94	.265	5	9	2.04	.278
72	2	43	88	2.93	.437	50	94	2.56	.370
	3	6	12	3.50	.531	3	6	3.30	.425
96	3	41	100	4.59	.640	48	100	3.66	.508
120	3	16	94	5.07	.788	49	100	4.63	.647
	P	1	6						

P—pupae.

with M/10 phenylalanine. This concentration retards all processes but does not affect the fertility or viability of the adult flies. Heir, Graham, and Kline (1944) found that it requires a concentration of ingested phenylalanine more than three times as strong as M/10 to decrease the rate of growth in rats.

That phenylalanine is less effective than tyrosine in accelerating the rate of larval moulting is shown in Table 5. At a concentration of

TABLE 5

PERCENTAGE DIFFERENCES BETWEEN TESTS AND CONTROLS FOR THE FIRST LARVAL MOULT AT VARIOUS CONCENTRATIONS OF TYROSINE AND PHENYLALANINE

Conc.	Tyrosine	Phenylalanine
M/10	— 8 (sat.)	—54
M/100	+12 (sat.)	— 1
M/250	+13	—11
M/500	+ 6	+11
M/1000	+ 5	+12
M/2500	+20	+ 1
M/5000	+ 2	— 3

M/2500, tyrosine increases the rate by 20 per cent while phenylalanine is ineffective. This result is similar to that obtained by Hammett (1936) for *Obelia*. It does seem doubtful, however, whether there

is here any correlation between concentration and degree of effectiveness. Despite the insolubility of tyrosine it is likely that it is less toxic than phenylalanine just as was found in the case of *Obelia*. Since there is little possibility of any iodine in the culture medium used for these experiments it would seem also that groups in the amino acids themselves are responsible for their effectiveness in the acceleration of differentiation.

3. *Alanine*

Drosophila larvae will tolerate alanine at as high a concentration as M/25 with but slight evidence of retardation, but if M/10 alanine is used there is a definite decrease in rate of growth and emergence of flies does not occur until 12 days after egg laying. Just as for phenylalanine this higher concentration does not affect the fertility or viability of the adult flies. Alanine does not however retard growth so much as the same concentration of phenylalanine (Figure 1). The difference is only a matter of a few hours for there was 100 per cent

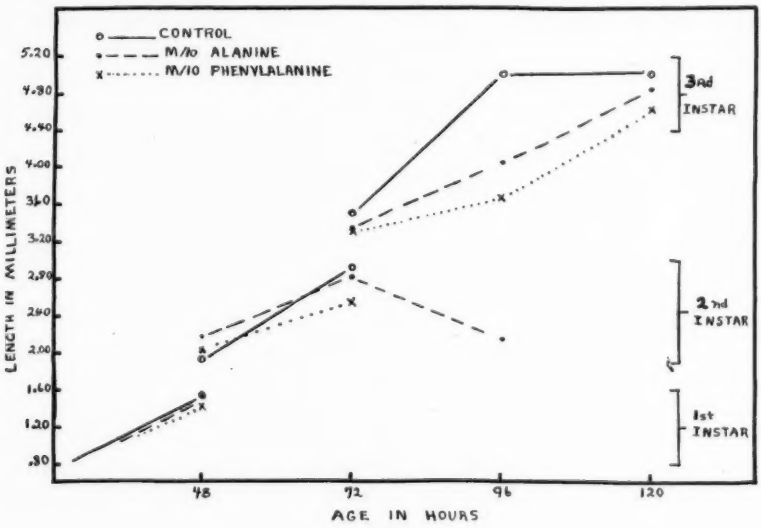

FIGURE 1

RATE OF GROWTH IN LENGTH OF DROSOPHILA LARVAE

TABLE 6

TABLE 6

AVERAGE LENGTH AND WIDTH IN MILLIMETERS OF LARVAE TREATED WITH ALANINE: AGE IN HOURS FROM MEDIAN HOUR OF OVIPOSITION

		Control				M/10				M/25			
Age	Instar	No.	%	Length	Width	No.	%	Length	Width	No.	%	Length	Width
48	1	69	62	1.56	.233	121	90	1.56	.237	27	51	1.59	.241
	2	42	38	2.17	.301	13	10	2.29	.313	26	49	1.86	.259
72	2	85	79	2.97	.433	52	93	2.85	.401	56	84	3.08	.461
	3	22	21	3.99	.573	4	7	3.34	.488	11	16	4.03	.563
96	2	0				14	13	2.15	.445	1	2	2.62	.375
	3	88	100	5.01	.726	90	87	4.09	.571	52	98	4.76	.704
120	3	35	88	5.04	.805	83	100	4.85	.683	46	100	4.82	.741
	P	5	12										

P—pupae.

TABLE 7

AVERAGE LENGTH AND WIDTH IN MILLIMETERS OF LARVAE TREATED WITH ALANINE: AGE IN HOURS FROM MEDIAN HOUR OF OVIPOSITION

		Control				M/500				M/100			
Age	Instar	No.	%	Length	Width	No.	%	Length	Width	No.	%	Length	Width
48	1	66	53	1.59	.241	43	48	1.61	.248	59	43	1.58	.261
	2	57	47	1.95	.272	47	52	1.98	.281	76	57	2.00	.279
72	2	73	76	3.07	.460	28	41	3.11	.482	101	72	3.03	.456
	3	23	24	3.62	.525	39	59	3.71	.536	40	28	3.87	.568
96	3	93	100	5.16	.823	47	100	4.94	.762	85	100	5.07	.762
120	3	45	100	5.11	.795	45	67	4.76	.771	52	100	4.75	.753
	P	0				22	33			0			
		Control				M/2500				M/100			
48	1	9	20	1.58	.238	8	13	1.54	.225	22	31	1.61	.242
	2	35	80	2.00	.278	52	87	2.03	.283	49	69	2.01	.270
72	2	24	41	3.05	.448	35	48	3.11	.473	37	60	3.08	.483
	3	34	59	3.79	.542	38	52	3.81	.552	25	40	3.63	.546
96	3	63	100	5.22	.789	59	100	5.20	.762	44	100	5.19	.776
120	3	37	83	4.89	.797	55	100	5.10	.790	34	100	4.90	.764
	P	8	17			0				0			

P—pupae.

emergence of imagoes in each case by the end of the 12th day from egg laying. The clue to the difference may lie in the fact that at more dilute concentrations alanine augments growth in length and width while phenylalanine has no such effect.

Table 6 also presents some evidence that M/25 alanine is on the border line between concentrations which retard and those which accelerate growth in length. Slight but consistent acceleration of growth in length and even to a greater extent in width is characteristic of larvae treated with concentrations of alanine between M/100 and M/2500 as shown in Table 7. Below a concentration of M/2500 there was no significant difference between test and control for any expression of growth.

Table 7 shows that there is significantly faster moulting in the range from M/500 to M/2500. Table 8 makes this point clearer by tabulating the percentage differences between tests and controls. The

TABLE 8

PERCENTAGE DIFFERENCES BETWEEN TEST AND CONTROL LARVAE AT THE FIRST AND SECOND INSTARS AND AT PUPATION FOR ANIMALS TREATED WITH ALANINE

Conc.	1st moult	2nd moult	Pupation
M/100	—11	— 9	—17
M/250	+ 7	+11	0
M/500	+ 6	+25	+33
M/1000	+10	+ 4	0
M/2500	+ 7	— 7	—17

effective concentration is M/500 where the acceleration is continued and augmented so that the larvae pupate significantly sooner.

These data differ from those obtained with the highly saturated tyrosine media where apparently the specific process of moulting is inhibited while increase in mass is accelerated. Addition of small concentrations of yeast to the basal medium would result in exactly the same condition as addition of M/500 alanine. It is more than likely that alanine then does not play a specific rôle in furthering differentiation as measured by early moulting, but that it acts merely as an additional supply of material for increased general growth or that it acts in some specific way to augment general growth.

Alanine is known to act in a specific manner in certain cases. Hauschka (1944) found it exerted a protective inhibition against the differential destruction of tissue by hexenolactone. It is also

known to accelerate hatching of mosquito eggs (Gjullin, Yates, and Stage, 1939). Regardless of interpretation, these results agree with those of Hammett that alanine plays some part in general tissue growth.

Summary

1. Larvae of Drosophila will tolerate the addition of M/10 concentration of each of the amino acids, tyrosine, alanine, and phenylalanine to the basal medium without evidence of actual toxicity.

2. Slight retardation of growth was produced by the highly saturated tyrosine concentration.

General retardation of growth was produced at M/100 phenylalanine and at M/10 alanine.

3. All three acids increase the rate of larval moulting. Tyrosine is more effective than phenylalanine but like phenylalanine produces the acceleration without any increase in width or length. Early moulting of alanine-treated larvae is accompanied by an increased rate of growth in width.

4. These results are interpreted to mean that tyrosine and phenylalanine are specifically concerned with differentiation while alanine acts to augment general mass increment.

5. The effective concentrations for tyrosine are between saturation and M/2500; for phenylalanine between M/500 and M/1000; for alanine between M/250 and M/1000.

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INTEGRATED PROGRAMS FOR GROWTH SYMPOSIA

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The Journal GROWTH, the *Growth Symposia,* and the Society for the Study of Development and Growth all stemmed from the apparent need for integrating investigation and thinking in the field of growth.

Even a most casual skimming of any number of BIOLOGICAL ABSTRACTS or CHEMICAL ABSTRACTS cannot but impress upon one the considerable attention that is being paid to this most important property not only of living things but also of things in general. And even a slightly thoughtful reading of the papers which are therein abstracted shows how restricted is the outlook of many workers regarding the potential ramifications of their particular findings, and how the admittance of other disciplines than the one used might help clarify some of the matters which lead them to futile verbifage.

The fact that the Journal GROWTH has survived for nine years and is going into its tenth year with papers of increasing authority; the fact that the growth symposia are becoming increasingly interesting to more workers; and the fact the Society for the Study of Development and Growth is steadily increasing its membership; all indicate that these three media for an attempt at integration are serving a useful function.

But there is nothing so good it cannot be improved. The Journal GROWTH could be strengthened by its sponsors—the Society for the Study of Development and Growth—sending more of their work to be published therein, and getting more subscribers therefor.

The Society for Study of Development and Growth could be strengthened by encouraging more of the younger workers to belong.

And the *Growth Symposia* could be increased in usefulness by a series of programs grounded in the integrated fundamentals of the property.

It is apparent that the four principle attributes of growth as of

other activities are: direction which is comprised in the science of genetics; substance which is comprised in the science of chemistry; state of substance which is comprised in the science of physics; and form which is comprised in the science of anatomy.

Who will deny that the progress of growth is the integrated expression of direction, change in substance, change in state of substance; and change in form?

And who is there who does not recognize that direction is expressed through substance; that state of substance is the property of the substance through which direction is expressed; and that form is the product-expression of direction expressed through substance and state of substance?

How then can any aspect of growth be comprehensively conceived unless through integration of its genetic, chemical, physical, and morphological attributes?

And what are these aspects of growth? For the present it is convenient to consider five as principle. These are Initiation; Proliferation; Differentiation; Organization; and Constructive Substance Increase.

Each may be taken to be subtended by its own particular brand of chemical reactions, participants, and determinants. And all are integrated through the inter-reactions of the chemical processes of one with those of the others.

Each aspect has also its own genetic determinants. In each the state of substance is peculiar. And form is a denominator of each.

But how many embryologists, morphologists, or anatomists are knowing of the contribution of genetics, chemistry, or physics to the forms they record? And how many biological physicists are knowing of the contribution of genetics, chemistry, and form to the states they study? And how many chemists are knowing of the part played by genetics, physics, and morphogenesis in the reactions they observe? And how many geneticists are knowing of the contribution of chemistry, physics, and even form to the expressions they observe? Yet no form, no state, no reaction, no expression of direction exists but what has genetic, chemical, physical, and anatomical attributes.

These things being so it would seem as if a series of Growth Symposia with programs grounded in these fundamentals would be most useful to students of growth interested in having for themselves a

picture of growth as an integrated compound of attributes and aspects rather than a picture of growth as a compartmentalized property of living.

Towards the attainment of this aim programs could be made up wherein the genetics, chemistry, physics, and morphology of each aspect were illuminated and discussed.

Thus one program could deal with the genetics, chemistry, physics, and morphology of constructive substance increase. Another could be given to initiation from these points of view. Another to proliferation. Another to differentiation. And another to organization.

Conversely a program could be given over to the genetics of initiation, proliferation, differentiation, organization, and constructive substance increase. One could take up the chemistry of these several aspects of growth. One to the physics. And one to the morphological changes thereof.

The morning session could be devoted to presentation of available knowledge on the particular aspect. With observations made by the leader as illustration. The afternoon session could be one of discussion, inquiry, development and exploration of concepts, and notation of the vast areas of ignorance wherein fruitful work needs doing.

And the whole symposium could be wound up on the last day with an attempt to integrate present knowledge into a working concept from which advances into terra incognita would start.

It is quite well recognized that such programs would be a radical departure from those usually performed. That their success would depend upon the insight of the chosen leader to pick out only the most useful data for presentation; his awareness of the fact these data, whether they be genetic, chemical, physical, or anatomical, are inextricably interwoven with data of the other attributes; and his awareness of the fact that the data, whether they be of initiation, proliferation, differentiation, organization, or constructive substance increase are in intimate contact at their edges with those of the other aspects. His function in the morning would be to give the evidence. From all pertinent work his chance at expressing himself would come in the afternoon when he takes part in the exploratory discussion. His acclaim would not come from recital of his own investigations as much as from the adequacy with which he instructed the group in the subject of the program!

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BOOK REVIEW

(Samuel Brody. Bioenergetics and Growth. New York: Reinhold, 1945.)

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From now on the caliber of any one claiming to have knowledge of growth will be judged by whether or not he is acquainted with this work of Brody's. These thousand pages packed with charts, tables, illustrations, references to some three thousand investigators and close to ten thousand papers, and the scholarly analysis and comment of the author, mark the product as a classic.

At long last we have in this study, not only an integration of energy expenditure in growth and maintenance; but also an advanced treatise on biological chemistry. Which definitely is needed. What is more we have a presentation which though centered around a way of working, weaves into interpretation of the results obtained therefrom, concepts derived from all the basic attributes of living things, viz., direction as comprised in the science of genetics; substance as comprised in the science of chemistry; state of substance as comprised in the subject of physics; and form as comprised in the science of anatomy.

Brody is clearly a scholar. For who but a scholar would aptly quote Woodger, Bertrand Russell, Pareto, Anacreon, Whitehead, Thomas Mann, Plato, Bacon, Ethel Barrymore, Will Rogers, and many other recognized and unrecognized thinkers and commentators on the human comedy.

It is a competent, efficient, comprehensive, practical, philosophical job. It is competent because it presents the data fully, concisely, logically. It is efficient because the reader can find what he wants clearly stated. It is comprehensive because it not only thoroughly covers the central theme; but also the ramifications thereof. It is practical because it integrates maintenance, growth, and the products of growth

with the cost thereof. And it is philosophical because it indicates how bioenergetics and growth are integrated with all our social, economic, political, military, and related problems. And does it calmly with no drum- or breast-beating; no belaboring of any pet thesis; but just simply and convincingly. This man is a scientist. And he has written a book for those who would be scientists; and those who would know of science as it can be.

For those who must know what is in a book before giving up $8:50: "the original material is on energy and nitrogen metabolism—including some bioenergetic methods—, on energy efficiency of productive processes—, and on various aspects of growth—, aging—, which we consider as inseparable from growth—, on the interrelation between part and whole—which we consider inseparable from the relation of energy metabolism to size—, and on physiological time and equivalence of age—. —the factors influencing efficiency were analyzed, such as the influence of plane of nutrition—, metabolic catalysts, including enzymes, minerals, and vitamins—, hormones—, season—, time of day—, temperature—, and nutritional balance—. Likewise the factors influencing profit were discussed with special reference to plane of nutrition—, body size—, productive plane—. The influence of the immediate efficiency and profit on longevity and fertility. —The theoretical feature of this book is the attempt to generalize large bodies of cumbersome data by simple rational but broadly inclusive equations."